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Review Series Article

Clinical and Preclinical Photodynamic Therapy

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Photodynamic therapy (PDT) is a treatment modality that utilizes a photosensitizing drug activated by laser generated light, and is proving effective for oncologic and nononcologic applications. This report provides an overview of photosensitizers, photochemistry, photobiology, and the lasers involved in photodynamic therapy. Clinical and preclinical PDT studies involving Photofrin and various second generation photosensitizers are reviewed.

Key words: photodynamic therapy, photomedicine, photosensitizers, Photofrin, lasers, tumor, oxidative stress

INTRODUCTION

Medical interest in the cytotoxic responses of photosensitizers has been recorded as early as 1900 [1–4]. However, the synthesis of hematoporphyrin derivative (HPD), a complex porphyrin mixture with reported tumor-localizing properties, by Schwartz in the 1950s [5], can be regarded as the beginning of modern photodynamic therapy (PDT). In the following years, experimental and pilot clinical studies evaluated hematoporphyrin and HPD for both diagnosis and therapy of malignant tumors [6–11]. Pioneering efforts in clinical HPD photosensitization were made by Dougherty [12,13], whose reports of a series of cancer patients treated by this technique appeared from 1978 onward. In 1980, Hayata and coworkers [14] were the first to apply fiberoptic endoscopic laser irradiation to treat early endobronchial lung cancer with PDT. Early studies, being anecdotal, tended to vary treatment conditions, but by the late 1980s, investigators using PDT for malignancies of the lung [15], esophagus [16], and bladder [17] were documenting staging, dosing, and tumor response with a goal of achieving standardization of this relatively new therapy.

In the past 15 years, several thousand cancer patients have undergone HPD- or DHE-mediated PDT, although the majority have not been part of prospective clinical trials. At the same time, second-generation photosensitizers and improved clinical laser delivery systems have been developed. After the completion of Phase III randomized trials, many ongoing at present, the status of PDT in comparison with conventional oncology treatment modalities will be known. PDT is being integrated into multimodality regimens, with the distinct advantage that photosensitizer injection and laser irradiation can be repeated multiple times.

There are also a number of nononcologic applications in which PDT is being evaluated. It is undergoing preclinical and clinical testing for its ability to inactivate viruses, to treat atherosclerotic lesions, and also to treat skin disorders such as psoriasis and portwine stains.

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PHOTOSENSITIZER DEVELOPMENT

Classes of Photosensitizers

Most clinical PDT experience comes from using the porphyrin variants, HPD and DHE. The active components of HPD were identified by Dougherty et al. [18] to be dihematoporphyrin ethers and esters (DHE). The commercial preparation of DHE, known as porfimer sodium, or Photofrin, contains <20% of inactive monomers and >80% of the active porphyrin dimers and oligomers. However, Photofrin remains a complex mixture with inherent variability, and it has the further limitation of weak light absorption at wavelengths above 600 nm. In addition, Photofrin has the side effect of causing prolonged cutaneous photosensitivity. These properties provided incentives for developing new photosensitizers. The next generation of clinical photosensitizers ideally will provide rapid plasma and tissue clearance, enhanced tumor to normal tissue selectivity, comparable photoactivation efficiency, and superior light absorption of visible red and near infrared light. Theoretically, these developments will lead to more selective treatment of large malignant lesions than is currently possible with Photofrin-mediated PDT.

A growing number of second-generation photosensitizers are being synthesized, which can be activated at wavelengths of light >650 nm. A nonexhaustive list of classes of compounds includes porphyrin and chlorin derivatives, purpurins, benzoporphyrins, phthalocyanines, and naphthalocyanines. The chlorins include many categories and are reviewed in detail elsewhere [19,20]. Chlorins are derived either by modifying a porphyrin (reducing one of the pyrrolic rings of the porphyrin macrocycle) or from chlorophyll as the starting material for synthesis. Purpurins are formally chlorins, since they have one reduced pyrrole group, as well as a fused five-membered isocyclic ring [21]. Benzoporphyrin derivatives are also formally chlorins, since they have a reduced pyrrole ring, as well as a fused six-membered isocyclic ring [22]. Phthalocyanines have been synthesized specifically for PDT [23]. They commonly incorporate a diamagnetic metal ion, usually zinc or aluminium, to enhance triplet photosensitizer yields and lifetimes in order to increase photodynamic activity [24,25]. Naphthalocyanines (absorption maxima 770 nm) have red shifted absorption spectra compared with phthalocyanines (maxima 670 nm). However, the properties of zinc and aluminium naphthalocyanines differ from their phthalocyanine counterparts in having high aggregation and photochemical instability, resulting in the naphthalocyanines being relatively photoinactive in vitro [24].

Second-generation photosensitizers undergoing clinical investigation include benzoporphyrin derivative mono-acid ring A (BPD-MA), mono-aspartyl chlorin e6 (NPe6), meso-tetra(hydroxyphenyl)chlorin (mTHPC), tin etiopurpurin (SnET2), and 5-amino-levulinic acid (ALA). The structures of these compounds and DHE are shown in Figure 1. Light at 650 nm is used to activate mTHPC, 660–665 nm is used to activate the chlorin and purpurin derivatives NPe6 and SnET2, and 690 nm light to activate BPD-MA. ALA is a precursor of protoporphyrin IX (PpIX) in heme biosynthesis, and endogenous PpIX produces effective photosensitization when activated by 630 nm light.

Tissue Distribution Studies

Considerable information on porphyrin tissue distribution has been obtained from preclinical animal studies [26–29], in addition to pharmacokinetic studies in humans [30–32]. Following intravenous injection, DHE has a biphasic plasma clearance in humans; an initial elimination half-life of 12–22 hours and a second half-life of 5–6 days have been reported [31,32]. The maximal therapeutic ratio for DHE between tumor and normal tissue varies between 24 and 96 hours. Many second-generation photosensitizers, such as NPe6 and BPD-MA, have a more rapid rate of clearance [33,34]. Consequently, photosensitizer injection and laser irradiation can be performed on the same day. DHE and NPe6 are primarily excreted unchanged through the feces, whereas BPD-MA is metabolized to an inactive form prior to excretion through the feces [27,33, 34]. Animal studies show that organ retention of these drugs is most persistent in reticuloendothelial tissues, such as liver, spleen, and kidney [26,33]. Levels in these tissues exceeded tumor levels at all time intervals after drug administration. Adrenal glands, pancreas, and bladder also retain high amounts of DHE. Skin and muscle take up relatively low levels of porphyrin and normal brain tissue has minimal uptake [27,28].

Transport in the blood of hydrophilic photosensitizers (hematoporphyrin monomers, tetrasulphonated porphyrins, and phthalocyanines) is mostly via albumen and globulins. These sensitizers localize in the stroma of tumor, vascular, and normal tissue. More hydrophobic photosensitizers (hematoporphyrin oligomers, mono and un-

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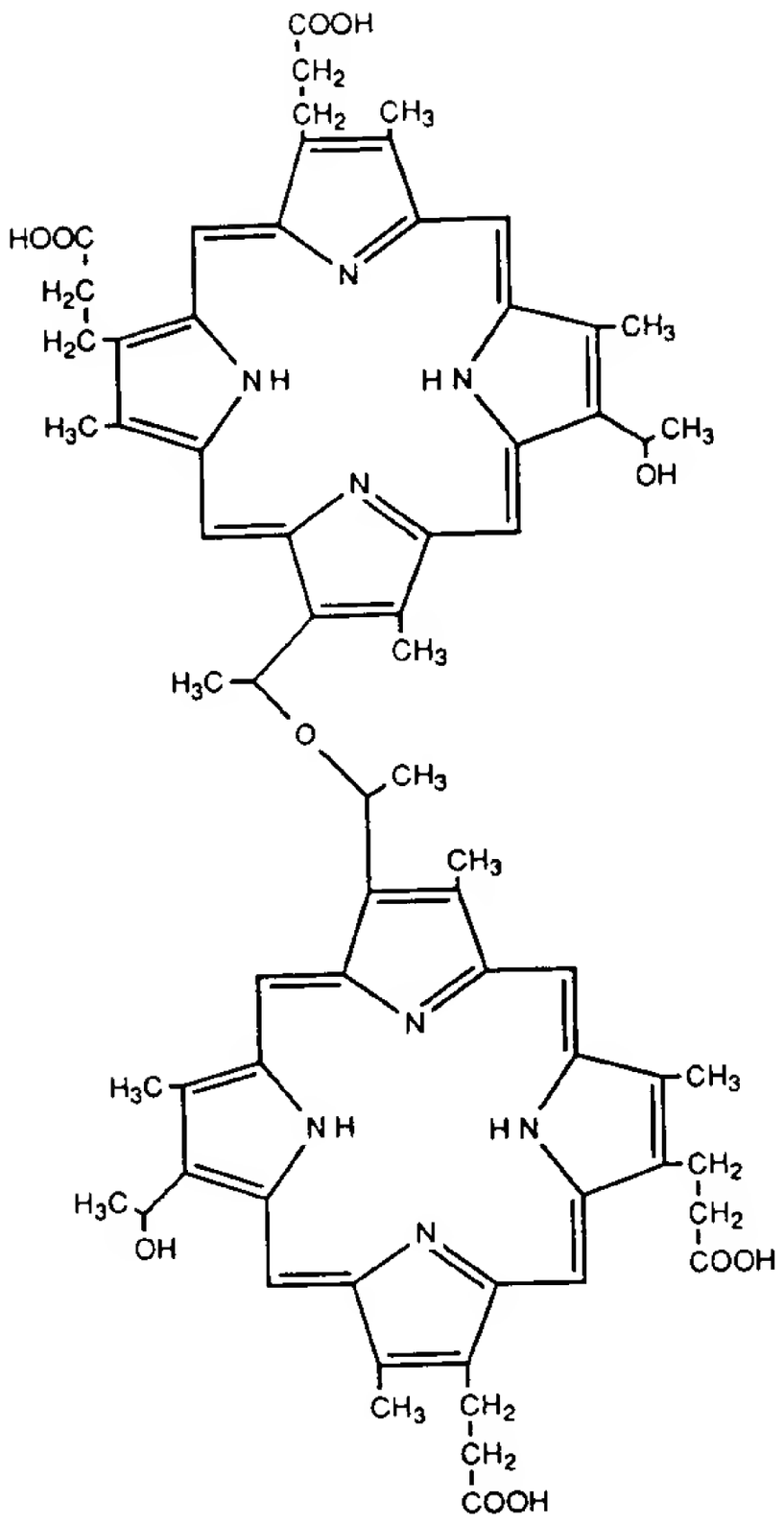

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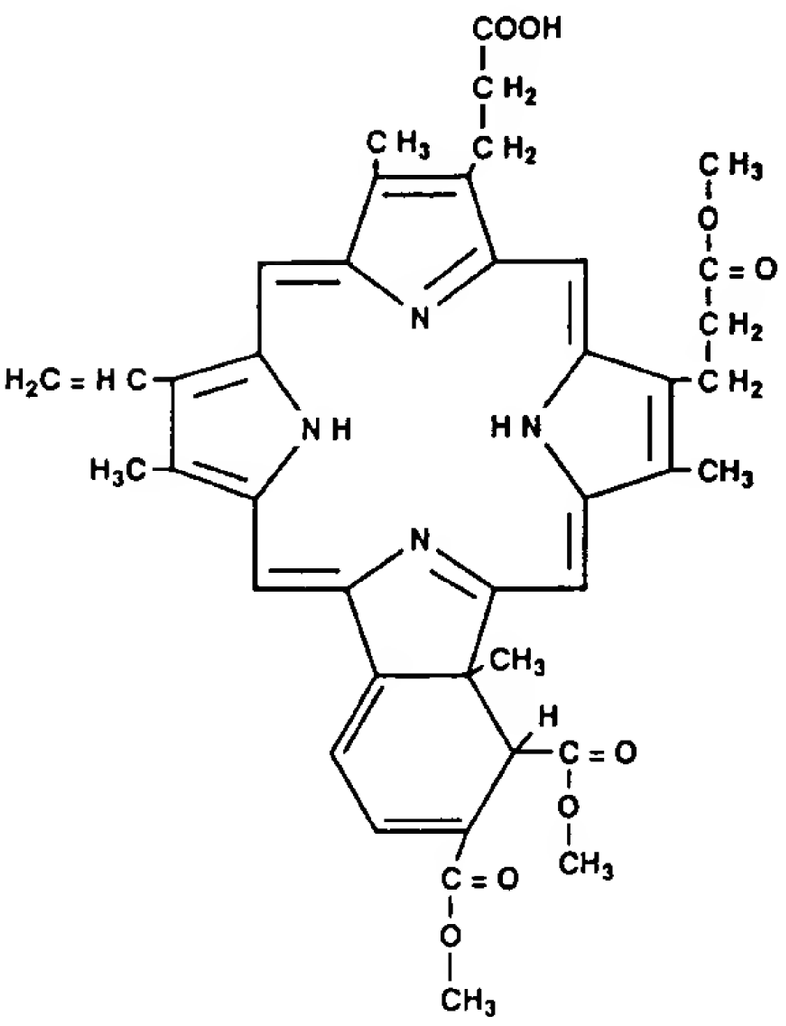

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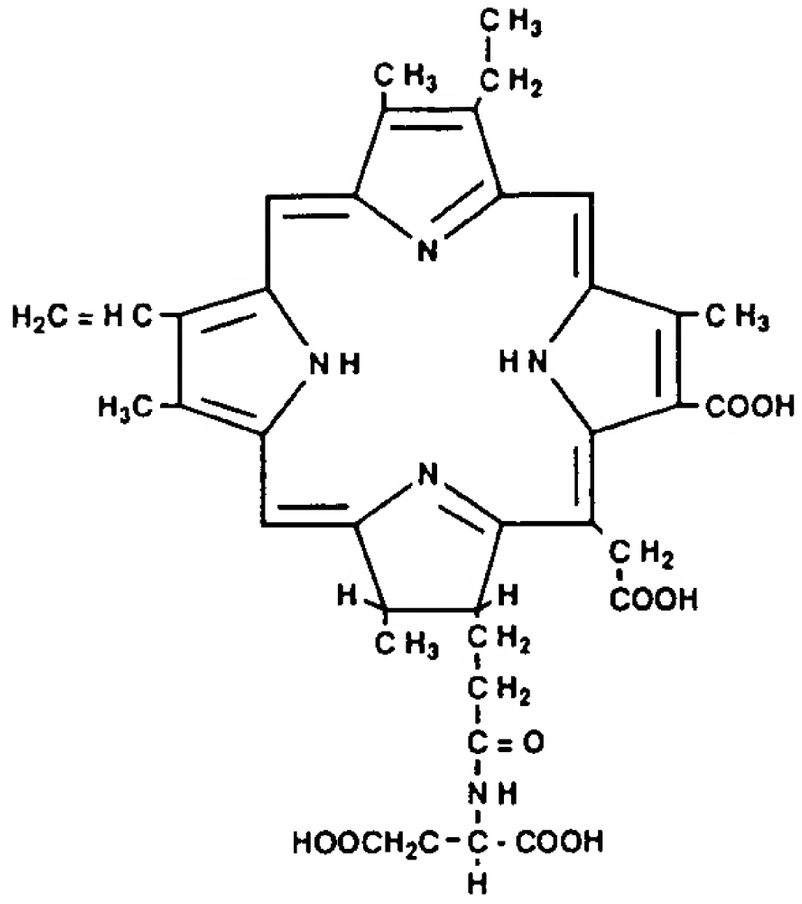

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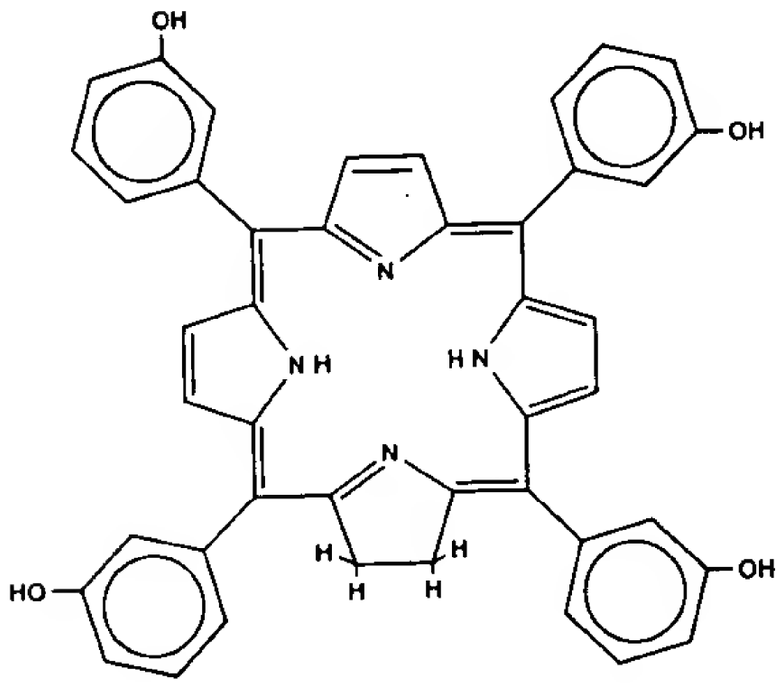

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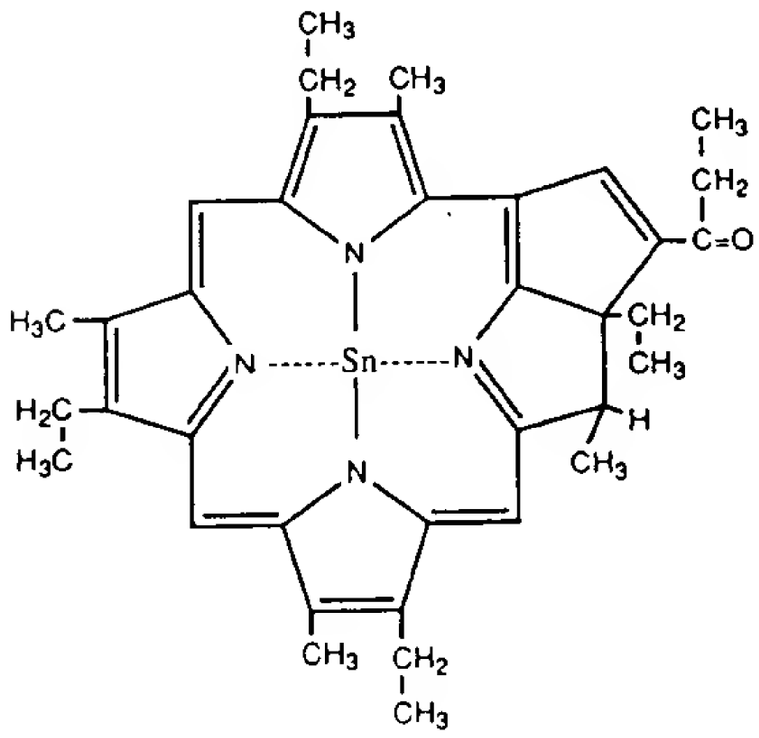

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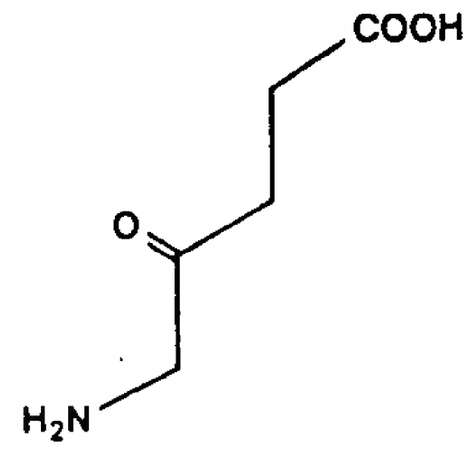

Fig. 1. Chemical structures for (**A**) dihematoporphyrin ether, DHE; (**B**) benzoporphyrin derivative monoacid ring A, BPD-MA; (**C**) mono-aspartyl chlorin e6, NPe6; (**D**) meta-tetra (hydroxyphenyl) chlorin, mTHPC; (**E**) tin etiopurpurin, SnET2; and (**F**) 5-amino-levulinic acid, ALA.

substituted phthalocyanines) are preferentially incorporated in the lipid portion of plasma lipoproteins [35]. Dyes with affinity for low density lipoproteins (LDL) are taken up by cells, at least in part, by receptor-mediated endocytosis. Lipoprotein-carried dyes are mostly deposited in endocellular loci, including mitochondria, lysosomes, and plasma membrane [35]. Otherwise, tightly aggregated dyes partly circulate as unbound pseudomicellar structures, which can enter cells by pinocytosis and localize in macrophages [35].

Photosensitizer Targeting

Approaches to improve the selective localization of photosensitizers in tumors involve binding the dye to targeting molecules such as antibodies, liposomes, and lectins [36,37]. The various conjugation strategies are described elsewhere [38,39]. The methods rely on the targeting molecule having high affinity for a tumor-associated antigen or receptor.

Plasma lipoproteins were found to play a major role in the in vivo transport of all classes of photosensitizers that are moderately or highly hydrophobic [40]. The low density lipoproteins (LDL) are of particular interest because they are recognized by specific receptors (e.g., the apo B/E receptor), which would result in LDL-bound photosensitizers being efficiently released to cells via apo B/E receptor-mediated endocytosis [41]. The process would favor cells that have a high content of LDL receptors, such as highly mitotic cells, including tumor cells, and endothelial cells [42]. In agreement with this, a correlation was seen between the extent of a photosensitizer's association with LDL and the efficiency of tumor targeting [40]. Therefore, various methods to enhance the LDL-mediated mechanism have been investigated, including formulation of photosensitizer in liposomes, lipid emulsions, inclusion complexes such as cyclodextrin, as well as preincorporation of the drug with LDL. Hematoporphyrin and zinc phthalocyanine incorporated into various liposomes show highly increased delivery to lipoproteins and high tumor uptake, compared with when administered in saline [40]. Photofrin prepared in LDL shows the same findings [40]. Benzoporphyrin derivative analogs, which naturally bind to the lipoprotein fractions when mixed with human plasma, have enhanced tumor uptake and tumor eradication when prebound to low density or high density lipoproteins [43]. BPD-MA is formulated in liposomes to achieve efficient tumor photosensitization [44]. ALA encapsulated in liposomes and injected into tumor-bearing mice induced higher endogenous porphyrin accumulation in the tumors and maximal tumor/skin ratios, compared with injection of free drug [45]. However, selectivity indexes cannot be extrapolated directly to humans, since interspecies LDL plasma concentration and receptor activity varies widely. Rabbits and dogs show more similar patterns of plasma lipoproteins to humans than do commonly used mice and rat models [42].

Photoimmunotherapy, also termed "antibody-targeted photolysis," is another targeting technique in which antitumor monoclonal antibodies (MAb) are used as carriers for photosensitizers [36,46,47]. In preparing the conjugates, the goal is to preserve activity of the MAb conjugate and maximize the number of photosensitizer molecules bound to the MAb. For example, a conjugate of MAb-dextran-chlorin achieves a higher ratio of photosensitizer to antibody than is obtainable with direct attachment. This conjugate was used to show that binding at high concentration to the plasma membrane was photodynamically effective and that the chlorin did not need to enter the cells [36]. In contrast, MAb delivery of most drugs and toxins requires internalization. The mechanism of photolysis appears to involve release of singlet oxygen by the conjugate, although the actual target sites of MAb-photosensitizer conjugates are unknown [36,47]. The cell membrane is probably a principal target of MAb-targeted singlet oxygen damage, and cytoplasmic constituents close to the membrane may also be affected. The technique can use a variety of photosensitizers (it is not necessary for the sensitizer to have tumor-localizing properties) and offers theoretical advantages, including sensitizer dose reduction and minimal or no skin photosensitivity, compared with systemic injection of free drug. The clinical role of MAb delivered photosensitizer is not yet defined, although animal models show in vivo effectiveness [47]. The biodistribution of the photosensitizer BPD, conjugated to a MAb specific for A549 human squamous cell carcinoma, was altered compared to injection of free BPD [48]. The results demonstrated that the sensitizer and antibody did not dissociate in vivo. In addition, the MAb-BPD conjugate showed specificity for the A549 tumor, in terms of its kinetics of tumor tissue accumulation of BPD compared with normal tissues.

A preliminary report of MAb-targeted photodynamic cancer treatment was documented in

three patients with advanced ovarian carcinoma, by Schmidt et al. [49]. A disulfonated zinc phthalocyanine was coupled by ester linkage to an anti-CA-125 antibody, since the patients all had elevated serum levels of the CA-125 tumor-associated antigen. The MAb-ZnPc conjugate was instilled in the peritoneum 72 hours before surgical tumor reduction and laser irradiation. After treatment, tumor cells were sampled for ultrastructural studies to detect signs of PDT damage.

For clinical application, there are several issues to address, in particular: (1) whether a large-size MAb-photosensitizer conjugate can reach cells in a solid tumor, (2) whether significant tumor cell antigen heterogeneity will arise, and (3) whether the host immune response will limit the technique. Methods to overcome each of these problems exist, such as use of small Fab or $(Fab')_2$ antibody fragments linked to the sensitizer and use of multiple MAbs to recognize different antigens. MAbs can be recognized as foreign proteins and become ineffective when neutralized. To decrease immunogenicity, it is desirable to use human antibodies and perhaps also to apply tolerance induction methods for reducing the immune response [50].

PHOTOCHEMISTRY AND PHOTOBIOLOGY

Type I and Type II Photochemistry

Upon absorption of a photon of light, a photosensitizer will be excited to a high energy singlet state. Singlet photosensitizer can decay back to its ground state, resulting in fluorescence emission. Alternatively, it can form triplet sensitizer, a slightly lower energy state, and longer lived excited species, by electron spin conversion in the process called intersystem crossover [51]. The fluorescent properties of photosensitizers have been useful for visualizing tumor localization and delineation of the malignant lesion. However, photodynamic action is dependent on intersystem crossover being the predominant process. The most efficient photosensitizers for PDT have a high triplet quantum yield and long triplet half-life. Triplet photosensitizer can undergo either Type I (electron or hydrogen atom transfer) or Type II (energy transfer) photochemical reactions. Transfer of energy to molecular oxygen is thought to be the primary photochemical reaction in porphyrin-mediated PDT. This results in the in situ generation of singlet oxygen ($^1O_2$) [52]. The scheme for type II photochemical reactions is shown in Figure 2.

Type I reactions probably occur also, porphyrins being most likely to undergo electron transfer processes with production of superoxide anions (O_2^-) [51]. Hydroxyl radicals and O_2^- have been detected during PDT reactions [53].

The highly reactive oxygen products of Type I and II reactions produce damage initially at the site of photosensitizer localization, due to their very short lifetimes in a biological environment. Unfortunately, it has been difficult to identify the initial target sites, because photochemical reactions can produce radical chain auto-oxidation and further oxidative reactions, leading to varying types of intracellular damage [51].

Cellular Targets of PDT

Subcellular sites of photodynamic damage include the plasma membrane and many organelle membranes, in particular the mitochondria [54]. Following DHE-mediated PDT, fluorescence and electron microscopy show immediate changes in mitochondria, with progressive swelling and structural disruption. Biochemical analysis has shown that PDT inactivates membrane-bound mitochondrial enzymes such as cytochrome C oxidase and succinate dehydrogenase, and inhibits respiration [55–57]. Damage to endoplasmic reticulum membranes is similarly observed, ultrastructurally and biochemically, with inactivation of acyl coenzyme A [58]. Plasma membrane depolarization and inactivation of transmembrane pumps, such as the Ca^{2+}/ATPase and the Na^+/K^+ ATPase, is observed following porphyrin PDT [59,60]. Chlorin, benzoporphyrin, and phthalocyanine photosensitizers cause damage to lysosomes, resulting in hydrolytic enzyme leakage [61]. It is probable that multiple sites and types of cellular photooxidation result from photodynamic treatment using the current photosensitizers, as none of the drugs are site-specific [54].

Damage to DNA has been demonstrated by measurement of single-strand breaks and sister chromatid exchanges, but this does not appear to be a critical determinant of cytotoxicity [62,63]. Cell sensitivity to DHE photosensitization was comparable in human fibroblast cells whether proficient or deficient in DNA damage repair [64]. The quantity of DNA–protein crosslinks (rather than DNA–DNA crosslinks) was thought to be a factor in the differential sensitivity to PDT in mouse lymphoma cell lines [65]. It was noted that one of the most sensitive lymphoma cell strains had a mutated thymidine kinase gene locus after PDT treatment [66]. However, mutation and car-

Type II Porphyrin Photochemistry

porphyrin + hυ	⟶	^{1}porphyrin	(absorption & excitation)
^{1}porphyrin	⟶	porphyrin + hυ	(fluorescence)
^{1}porphyrin	⟶	porphyrin	(nonradiative decay)
^{1}porphyrin	⟶	^{3}porphyrin	(intersystem crossover)
^{3}porphyrin	⟶	porphyrin + hυ	(phosphorescence)
^{3}porphyrin + $^3O_2$	⟶	porphyrin + $^1O_2$	(energy transfer)
$^1O_2$ + substrate	⟶	oxidized substrate	(photooxidation)

hυ = light quantum

^{1}porphyrin = singlet excited state porphyrin

^{3}porphyrin = triplet excited state porphyrin

$^3O_2$ = ground state oxygen (triplet state)

$^1O_2$ = singlet oxygen

Fig. 2. Type II photochemical reactions involved in the cytotoxic action of porphyrin PDT.

cinogenic transformation levels were measured as unchanged after a wide range of porphrin-mediated photosensitization doses [67].

Interestingly, PDT induces the expression of several types of stress proteins in cells, including heat shock proteins (HSP) and glucose-regulated proteins (GRP), although the specific response varies as a function of the photosensitizer and sensitizer incubation conditions [68–70]. Cells exposed to DHE and light, after a long incubation protocol to allow intracellular localization of drug, show induction of GRP78, GRP94, and hemeoxygenase (HO). NPe6 PDT and SnET2 PDT induce GRPs and HO, as well as HSP70 and HSP25. The induction of stress genes by PDT appears to be at the transcriptional level, but the complex problem of what target damage is responsible for induction of each stress gene is yet to be determined.

Apoptosis is also induced by PDT and appears to involve a signal transduction pathway originating at the cell membrane. Oleinick et al. [71,72] demonstrated characteristic DNA fragmentation, chromatin condensation, and activation of a constitutive endonuclease in phthalocyanine and porphyrin photosensitized cells. Inositol triphosphate (IP_3) release was measured as a result of phospholipase C activation by PDT. The pathway is thought to follow IP_3 release, rise in free intracellular Ca^{2+}, activation of phospholipase A_2, and subsequent release of arachidonic acid. One of the metabolic products of arachidonic acid presumably activates an apoptotic endonuclease. Importantly, apoptosis has also been identified in vivo as an early event in tumor shrinkage following DHE or phthalocyanine-mediated PDT [73]. The significance of apoptosis in the clinical PDT response compared with necrotic cell death is unknown.

Vascular Destruction Versus Direct Tumor Cell Kill

Experimental studies indicate that vascular injury plays a major role in tumor destruction following PDT. The in vivo response of porphyrin-mediated PDT is characterized by rapid onset of vascular stasis, vascular hemorrhage, and both direct and anoxia-induced tumor cell death. In a study examining perfusion of mouse tumors after

Photofrin PDT, tumor regrowth delay correlated with treatment protocols that cause the most severe reduction in tumor blood flow [74]. Vasculature destruction also appears to be the major effect following chlorin and phthalocyanine photosensitization, with tumor cell death occurring secondary to vascular shutdown [75]. Henderson et al. [76] used an in vivo/in vitro technique to demonstrate the time course of PDT events. In tumors removed soon after HPD PDT treatment, the cells were clonogenically viable, but viability decreased with longer intervals of sampling. Tumor cell death was occurring later from oxygen and nutrient deprivation, following early vascular injury.

Endothelial cells and macrophages are known to be particularly sensitive to photosensitization. Irradiation of sensitized mast cells and macrophages causes release of vasoactive inflammatory agents and cytokines, including prostaglandins, lymphokines, and thromboxanes [77, 78]. These inflammatory mediators seem to play an important role in the microvascular response to PDT, since administration of cyclooxygenase inhibitors not only inhibits their release, but also inhibits PDT-induced vascular damage and tumor destruction [79,80]. However, there does not seem to be any significant difference in photosensitivity between tumor and normal vascular endothelium [81].

It is likely that the mechanism of PDT tumor destruction in human tumors is not always the same as found using transplanted animal tumors. One reason is that spontaneous tumors have marked differences in vascular and stromal structures. It has been suggested that in the clinical situation, the vascular effects may be less responsible for tumor destruction than direct killing of tumor cells. An initial increase in blood flow can sometimes occur, seen in preliminary human tumor blood flow studies [82]. Also, direct cell kill effects might be underestimated from the mechanistic studies in animals. Histological evaluation of tumors following PDT shows clear demarcation of tissue necrosis, corresponding to depth of light penetration and not consistent with vascular occlusion causing cell kill [82]. Ultimately, the relative contributions from tumor cell and vascular photosensitization will depend to some extent on the time interval employed between drug injection and light irradiation and drug dose. The photosensitizer type is a particularly important factor due to their variation in clearance kinetics and tissue compartment localization [77].

Tumor Selectivity of PDT

PDT offers a degree of tumor selectivity with minimal systemic side effects. Factors contributing to selectivity include: (1) preferential photosensitizer localization in neoplastic tissues, and (2) precise laser light irradiation of the tumor region. The first factor cannot be relied upon using current photosensitizers to produce selective photodynamic treatment of the malignant lesion, since the amount of differential localization between tumor and normal tissue is highly variable. There are several theories regarding the mechanisms whereby photosensitizers can accumulate or be retained in neoplastic tissue more than in the adjacent normal tissue. It is probable that more than one mechanism is operating.

The majority of data on in vitro cell studies indicate that normal cells and cells of varying oncogenic potential take up similar levels of photosensitizer [83]. Cationic intramitochondrial dyes are an exception, capable of producing selective in vitro photolysis, due to increased dye incorporation by carcinoma cells [84]. Tissue physiology is clearly important, since Chan et al. [85] transplanted the same tumor (colorectal carcinoma) to different organs in mice and found significant variation in in vivo ClAlPc uptake. Henderson and Dougherty [82] suggest that simple pooling and retention of photosensitizer could occur as a result of the typically large interstitial space and poor lymphatic network characteristic of tumor tissue, in comparison with normal tissues having lower interstitial, higher vascular spaces [86]. The tumor localization properties of anionic dyes, such as hematoporphyrin derivatives and phthalocyanines, are thought to involve tissue factors such as low pH, and increased amounts of macrophage infiltration and newly synthesized collagen [83]. The density of lipoprotein receptors was proposed as a more specific mechanism for increased uptake, whereby LDL-bound photosensitizer rapidly enter neoplastic cells by receptor-mediated endocytosis [87]. However, uptake assisted by LDL binding is not the only explanation since protoporphyrin associates well with lipoproteins but is a poor tumor localizer. Several other dyes, such as TPPS and uroporphyrin, are reported to be good tumor localizers, although they associate poorly with lipoproteins.

The drug concentration ratio depends on the tissue. The highest tumor to normal tissue ratios of Photofrin have been reported in the brain, which might be due to a breakdown in the blood-

brain barrier at the tumor site [88]. In skin, the tumor to normal tissue ratio of Photofrin in rodent models is <2:1. However, human malignant skin lesions have shown more selectivity in treatment response than rodent models [82]. In reticuloendothelial tissues where uptake of current photosensitizers is high, there is no time interval that produces a useful ratio. Understanding the mechanisms for preferential uptake is mainly important for attempting to improve tumor targeting of sensitizers. Methods of targeting photosensitizers using carrier molecules or delivery systems may prove worthwhile as a means to increase tumor selectivity.

Combined Use of PDT and Hyperthermia

In general, the reason for using nonthermal power densities for photodynamic treatment is to exploit the potential selectivity of PDT by irradiating tumors at a time when the photosensitizer is retained in higher concentrations than the surrounding normal tissue. This allows undefined tumor margins to be lasered more safely. Clinically, combined hyperthermia and PDT tend not to be employed, although simultaneous treatment could be achieved simply by using higher dose rates of light during PDT.

From experimental studies, hyperthermia (HT) has been proposed to be a useful adjunct to photodynamic therapy for some applications, since the two treatments can be synergistic. In vitro and in vivo experiments indicate that the therapeutic response is synergistic or superadditive only within a short window, when HT is applied before, during or immediately after PDT [89,90]. The following mechanisms have been suggested for the synergistic response that follows the specific treatment sequence of PDT followed by HT. The rapid vascular destruction caused by PDT can hinder heat dissipation by blood circulation and increases the temperature differential between tumor and normal surrounding tissue [91]. At the cellular level, PDT and HT may have targets in common, particularly membranes. The proteins of the plasma membrane and mitochondrial membranes undergo structural transitions at hyperthermic temperatures [91]. Despite PDT and HT having similarities in their subcellular targets and denaturing effects on proteins, there is no evidence that the two modalities share mechanisms of cytotoxicity. Cross resistance to PDT is not observed in temperature resistant murine fibrosarcoma cell lines [92].

Heat applied before PDT may be a less effective combination in vivo, since the vascular modifications due to HT, such as hemorrhage, could drastically decrease light penetration in the tumor [91]. Heat-induced capillary collapse could significantly decrease oxygenation in the tumor microenvironment, which would theoretically impair the efficiency of photodynamic action [91].

Advocating against combined PDT and HT to obtain improved tumor control, injury to normal tissue can also be increased as a result of vascular effects common to both treatments. An experimental model showed that combined treatment of PDT followed by HT required an interval of more than 21 days between modalities to minimize normal skin necrosis [93].

LIGHT IRRADIATION

Laser and Nonlaser Sources

Incandescent filament (tungsten) and arc (xenon, mercury) lamps were used in early clinical PDT studies. It seems likely that nonlaser sources of light will continue to have a useful role, even though they supply relatively broad spectrum light. Lasers have become the standard light source for most clinical PDT applications largely because the laser beam can be efficiently coupled into single optical fibers, ideal for inserting in flexible endoscopes and for interstitial use.

Laser light is monochromatic, and the wavelength chosen depends on the specific photosensitizer and application. The absorption spectrum of DHE includes a high Soret band absorption (370–410 nm) with progressively smaller Q bands (505, 540, 580, and 630 nm) [94]. The 514 nm output of the argon laser is suitable for PDT applications where tissue penetration requirements are minimal, such as in certain cancers of the peritoneal cavity or bladder. Although Photofrin absorption is minimal for 630 nm light, this wavelength is routinely used for Photofrin-mediated PDT because light penetration in tissue is greater than at the shorter wavelength Q bands [95]. The argon ion laser-pumped dye laser has been the most widely used laser system to produce 630 nm light. In the visible red spectrum, the choices of gas and solid state laser with sufficient power for PDT treatment are limited. The gold vapor laser (GVL) emitting at 628 nm can generate over 1W of power. Optically pumped dye lasers remain a popular light source for PDT, since single dyes can cover a significant range of wavelengths. The tunability is an obvious advantage of dye lasers over the GVL, since the output wavelength can be al-

tered accordingly to suit new drugs with varying absorption properties.

Argon ion laser-pumped dye laser (ADL). This has been the most widely used light source for clinical PDT and emits continuous wave (CW) light. Medical ADL systems have minimized the requirement for precise optical alignment of the dye laser. Argon lasers are termed small frame (7–10 W) or large frame (20–25 W) and generate 1–2 W or 3–4 W of red light, respectively, out of the dye laser. This light can be coupled with 80–90% efficiency into single 200–400 μm fibers. Rhodamine B is a relatively stable dye with long-lasting lifetimes, most commonly employed in the ADL to obtain 630 nm light; DCM (4 dicyanomethylene-2-methyl-6-dimethylaminostyryl-4H-pyran) and Kiton red are other dyes of choice for obtaining light of this wavelength.

Gold vapor laser (GVL). The GVL produces a pulsed output at 628 nm. Compared to dye lasers, GVL are tolerant to misalignment and easy to operate. The laser pulse duration is typically 50–100 nsec and pulse repetition frequencies tend to be in the range 4–20 kHz for commercial systems. Average output powers range from 1.5 W to 9 W. The fixed wavelength output of 628 nm matches Photofrin absorption, although it would be possible to use this laser for new photosensitizers, by converting the plasma tube to a copper vapor laser for pumping a tunable dye laser.

Copper vapor laser-pumped dye laser (CVDL). The copper vapor laser is also a pulsed system with pulse structures similar to the GVL. The output from the copper laser at 510 and 578 nm would be useful only in surface PDT treatments. Its high pulse repetition frequency and high average pulse power make it suitable as a pump laser for dyes with emission in the red and near infrared. A negative feature of this pulsed laser output is a large beam divergence, requiring a larger diameter fiber (1,000 μm) for light delivery. Like the ADL, its most important characteristic is its tunability, particularly useful when new drugs are approved.

Excimer laser-pumped dye laser (EDL). This laser system is widely used by Japanese clinicians in their Phase III registration studies using Photofrin. XeCl or XeF gas is excited to produce UV line output, which is then used for pumping rhodamine or DCM dye to produce 630 nm light. The excimer laser is a high power pulsed laser, capable of megawatt peak output of 10–100 ns pulse duration. The EDL has a low repetition rate (maximum 80 Hz).

Solid state lasers. The neodymium:YAG (Nd:YAG) laser emitting at 1,064 nm or frequency doubled to emit at 532 nm has applications in surgical specialties, the wavelength of choice depending on 1,064 nm light having excellent penetration properties through hemoglobin, whereas 532 nm light does not. With regard to suitability for PDT, frequency doubled operation can be used to pump a dye laser resulting in tunable pulsed laser output. A combination system has been assembled intended specifically for this application, in which a KTP doubled Nd:YAG laser (line output at 532 nm) is used to pump a dye laser to emit light at 630 nm. The average power is 3–4 W from the KTP-dye laser system. The repetition rate is 25 kHz and the pulse width is 470 nsec. Alternatively, Nd:YAG has several minor lines, such as 1,318 nm, which can be frequency doubled to provide 659 nm light.

Tunable solid state lasers have advanced considerably in the past 5 years and are being tested experimentally for PDT use. They can only generate far-red/near infrared light, so they are potential laser sources for matching to second- and third-generation photosensitizers. The titanium:sapphire (Ti:Al_2O_3) laser has three sets of optics to cover the wavelength range 690–1,100 nm; the alexandrite lasers have a working range 720–800 nm.

Diode lasers. Major progress in the use of semiconductor laser diodes for PDT has been gained by making phased arrays of the output beams from multiple low power diodes to make a sufficiently high power coherent beam. Diode lasers are a portable size and represent convenient light sources. Most development is on the GaAlAs diodes, usually operating in the wavelength range of 780–850 nm with 1–5 W output. Diode laser systems emitting at 660–700 nm have been developed, but the power output is lower. Diode arrays have considerable potential for PDT involving current sensitizers (NPe6, BPD) and new sensitizers with absorption in the far-red region. The quality of the output beam is relatively divergent compared to the other laser systems described, making it more difficult to couple to fiber optics.

Comparison of CW and Pulsed Lasers for PDT

There are few prospective studies comparing CW and pulsed laser systems for PDT. In general, it has been demonstrated that both types of laser light can be used for therapy. There is insufficient information for the new laser systems being in-

troduced and, therefore, further evaluation will be required in this regard. Controlled studies are required to determine biological equivalence for the EDL and solid-state lasers with the ADL, in terms of PDT efficacy and safety. Pulsed lasers operating at very high repetition rate represent a quasi-CW mode. Differences in effects may be expected with pulsed lasers that have a high peak power per pulse.

Several studies have been conducted to directly compare the ADL (630 nm light) and GVL (628 nm) [96–98]. One experimental study used a cell culture and a murine tumor response assay [96]. Both laser systems were tested using 400 mW output (average pulse power of 400 mW), coupled to a 400 μm fiber to create a 1 cm diameter spot. The GVL had a 50 ns pulse width and repetition frequency of 10–14 kHz. The lasers were equivalent in in vitro cytotoxicity and in tumoricidal efficiency. For clinical usage, which generally required ~1 W of power, the GVL was easier to operate [97]. Output needed to be coupled to a 600μm diameter fiber (compared to 200 μM with the dye laser), which can be a disadvantage if the large, less flexible fiber reduces the maneuvreability of endoscopes. Otherwise, light applied continuously or in a pulsed mode appeared to make no difference to the results of patient PDT treatments. A recent study compared the ADL and GVL for treatment of virally induced papillomas in rabbits [98]. The GVL produced a faster rate of initial response following PDT, but ultimately there were no differences in overall cure rate, histology assessment, or viral DNA analysis from involved tissues using either laser system.

Barr et al. [99] compared three lasers for photodynamic effectiveness using normal rat colon as an in vivo model and aluminium sulphonated phthalocyanine as the photosensitizer. An ADL system (DCM dye), a 10 kHz repetition CVDL (Oxazine72/Rhodamine G dye), and a 5 Hz repetition flashlamp-pumped dye laser (cresol violet dye) were evaluated. Each laser was tuned to emit 100 mW at 675 nm, coupled to a 200-μm fiber. The ADL and CVDL were comparable at producing damage, measured as the radius of necrosis in histology sections. The CVDL pulses were 40 ns width and 10 mJ energy. The flashlamp-pumped dye laser produced 2 μs, 20 mJ pulses, and failed to produce a photodynamic effect. The most likely explanation for the ineffectiveness of this laser was that the higher energy, microsecond pulses produced saturation of the phthalocyanine. Specifically, the pulse energy was able to pump most of the ground state photosensitizer to an excited state and deplete the ground state population, so that subsequent pulse energy is not used efficiently. Saturation pumping is a common process for phthalocyanines because they have a high absorption coefficient. However, the flashlamp-pumped dye laser was also found to be ineffective for PDT mediated by HPD in a murine tumor model, despite HPD having a lower absorption coefficient and lower potential for saturation [100].

A direct comparison has also been made between the ADL and the pulsed KTP-pumped dye laser [101]. Both dye lasers were tuned to emit 630 nm light and the output coupled to a 200 μM fiber. The lasers were tested over the range 0–400 J/cm^2 using a power density of 75 mW/cm^2. They were shown to be biologically equivalent in several types of experimental systems, including in vivo tumor response, murine skin photosensitization, and in vitro cytotoxicity. Furthermore, tumor temperature levels during laser exposure, amount of DHE photobleaching, and induction of cellular stress protein synthesis were observed to be identical using either laser system.

Laser Dosimetry and Delivery

The clinical effectiveness of PDT for solid tumors depends in large part on the transmission of adequate light throughout the tumor tissue. The aim is to disperse low power light uniformly, either over the surface area or into the volume of tissue, to initiate the photochemical process without inducing side-effects, such as thermal damage of adnexal structures. This is in contrast to surgical laser treatments, in which light is focused for cutting, coagulating, or photoacoustical effects. In PDT, further requirements of the delivery systems are to make them: (1) compatible with other clinical instrumentation, such as endoscopes and stereotactic devices, (2) to incorporate light output monitoring and dosimetry devices, and (3) to tailor the light spatial distribution to match the tumor shape and size in each patient [102].

The light dose chosen for PDT depends on the size, location, and type of tumor. Using Photofrin and 630 nm light, typical radiant exposures are 25–300 J/cm^2 for surface treatment and 100–400 J/cm for intersitial applications, with maximum irradiances of 200 mW/cm^2 or 400 mW/cm, respectively [103]. This has generally been attained using laser sources having an output

power of 1–2 W. However, higher power lasers (at least 5 W) may be required during intracavitary PDT, involving treatment of large surface areas in pleural and peritoneal cavities.

Power requirements are not likely to be much less with second-generation photosensitizers either, since the rationale for these is to allow treatment of larger tumors by exploiting their higher extinction coefficient and longer wavelength activation. Another situation in which a higher light dose is required than normal is during differential photobleaching of photosensitizer in tumor and adjacent normal tissues [104]. The technique can potentially improve the therapeutic ratio of PDT and it involves significant photosensitizer dose reduction. The light dose needs to be increased more than proportionally to achieve equivalent photodynamic tumor destruction.

Laser delivery systems differ depending on the application. Rather than simply using an expanded laser beam from a bare fiber, more uniform irradiation is obtained by fitting a microlens to the fiber for forward surface illumination [105,106]. For treating thicker lesions and tumors within the body, the use of interstitial laser irradiation is required. The fiber can be directly inserted into the tumor mass, either by point insertion or inside a needle using a flat cut fiber tip, or by insertion of spherical and cylindrical diffusing tips. If several sites are to be irradiated, translucent nylon catheters can be surgically implanted for subsequent laser treatments.

The concept of "photodynamic dose" and contributing factors have been described by Wilson [107]. During patient follow-up, a wide range in tumor response is seen. Factors responsible for heterogeneity are speculated to include differences in photosensitizer uptake and light transmission within the tumor, and variation in tumor tissue sensitivity depending on cell composition, vascularity, and oxygenation. Techniques to measure light fluence within tissue, photosensitizer concentration, and tumor tissue oxygenation are being developed to assist patient PDT treatments.

Several workers [105,107] have identified the requirement for incorporation of light monitoring and dosimetry instruments into clinical delivery systems as the next essential step to gain information from each patient treated with PDT. Invasive and noninvasive devices will be able to provide real-time information during the laser procedure. Direct noninvasive measurement of drug concentration in a tissue can be based on quantitative fluorometry or reflectance spectrophotometry, although these only provide average values. Transcutaneous DHE levels in an animal model were measured using a hand-held fluorometer and showed a good correlation with fluorescence measurements of DHE in skin biopsy specimens [108]. Similarly, noninvasive measurement of local oxygen concentration can be made during treatment. Tromberg et al. [109] used transcutaneous oxygen electrodes in rabbits transplanted with VX-2 skin carcinomas. PDT using low light doses caused a reversible decrease in oxygen tension, whereas large fluences caused long-term irreversible hypoxia.

There are ongoing attempts to make in vivo measurements of singlet oxygen ($^1O_2$) production, by monitoring its luminescence emission at 1,270 nm, since $^1O_2$ is generally accepted as a key intermediate in the photodynamic effect [110]. It is thought that a minimum threshold level of $^1O_2$ (or photoactivated species) is required to produce tumor necrosis. So far, it seems in a cell or tissue environment, the extremely short lifetime of singlet oxygen (<0.5 μs) prevents reliable detection with present infrared detectors [111,112].

PHOTODYNAMIC THERAPY APPLICATIONS IN CANCER TREATMENT

Current Status of Clinical Photofrin PDT

PDT has been used to treat several thousand cancer patients as an investigational modality. Recently, Canada received Board of Health approval for the use of Photofrin-mediated PDT for treating superficial bladder cancer. In addition, The Netherlands has permitted licenses for treating lung and esophageal cancers with Photofrin PDT. Further regulatory submissions for a variety of applications have been made in Japan, Belgium, Germany, Denmark, and Greece. A product license for PDT specifies not only the photosensitizing drug, but also the laser type and the fiberoptic devices for producing and delivering the light [113].

The following Phase I and II trials are underway or near completion in the United States: for breast metastases, gynecological tumors, cutaneous cancers, Carcinoma In Situ (CIS), Kaposi's sarcoma, and papillomatosis, plus Phase I/II trials for intraperitoneal and intrapleural (intracavitary) PDT. Phase III trials in the United States, Canada, and Europe are evaluating Photofrin PDT for treatment of endobronchial lung cancer, esophagus, superficial bladder cancer, and prophylaxis of bladder cancer following transure-

thral resection (TUR) of tumors. Japan has Phase III clinical trials in progress for early stage lung, esophagus, gastric, bladder, and cervical cancers.

Clinical Studies of PDT Using HPD/Photofrin

This section reviews the current status of clinical PDT treatment using Photofrin (DHE) or its predecessor, HPD. Details are given for specific laser delivery systems designed for the specific cancer type. Clinical outcomes are mostly described as complete response (CR; no tumor present grossly and microscopically), partial response (PR; >50% decrease in all tumors treated), with the remainder of lesions representing progressive disease. Follow-up times vary in each study.

Endobronchial lung cancer. The lung cancer mortality rate remains high, despite increased screening and early detection. This disease is thought to be multicentric; patients have a high risk of developing another primary lung tumor even after complete resection of the original lesion [114,115]. This means that surgical treatment of initial early stage lung cancer has become as conservative as possible to preserve lung tissue. Surgical resection can be totally successful at removing the original lesion, but patients frequently have coexisting pulmonary or cardiovascular disease, making them a high surgical risk [114,115].

PDT represents a local therapeutic modality that can produce complete responses and cure of centrally located early stage endobronchial lung cancer [116,117]. Results from ~500 patients with this disease have been reported to produce complete and partial response rates ranging from 70–100% [118]. Superficial disease at the time of treatment is an essential factor for long-term effectiveness. PDT is useful for patients who cannot undergo surgery, as well as for palliation of advanced endobronchial malignancy. Patients with endobronchial tumor obstruction recruited in Phase III studies are randomized to receive either palliative Nd:YAG treatment or Photofrin PDT. Clean-up bronchoscopy is routinely scheduled 24–48 h after PDT to prevent complications of pulmonary obstruction, due to mucosal plugs and necrotic tissue.

McCaughan et al. [119] reported treatment of 31 patients (49 tumor sites) with endobronchial cancer using HPD and Photofrin PDT. All patients had been pretreated with or were unsuitable for conventional surgery, radiation therapy, and chemotherapy. An ADL system was used to supply the 630 nm light using a flexible bronchoscope, incorporating a biopsy channel. The results were promising in that 37% had a complete response and only 4% had progressive disease at 1 month after treatment.

By 1989, Kato et al. [120] had treated 165 patients with lung cancer by PDT, using an argon-dye laser and an excimer-dye laser as light sources. Forty patients did not have disease evident on chest X-ray, but endoscopically were classified as having early stage lung cancer. The majority of the 165 patients received additional surgery, radiotherapy, or chemotherapy, but a total of 26 patients (with 30 lesions) received PDT as the sole treatment. All lesions in the PDT-only group showed complete remission initially, with 16 patients remaining disease-free and three patients classified as 5-year "cures." Ono et al. [121] treated 36 patients with biopsy specimens positive for malignancies of the trachea and bronchus; again not all identifiable on chest X-ray. HPD was administered 72 hours before laser treatment under fiberoptic bronchoscope delivery. The range in response was a complete response with no recurrent disease in 16 patients and death of 20 patients related to the disease. Follow-up ranged from 37–109 months. Cortese [114] has treated > 60 lung cancer patients with PDT. Patients were not deemed suitable for this treatment if lymph nodes were known or suspected to be involved. Some of their patients were suitable for conventional surgery but received PDT as a first-line treatment instead. Twelve of 13 such patients demonstrated a complete response after one or two PDT sessions, and these were all superficial tumors. The one tumor treated that showed only a partial response was a bulky, exfoliative lesion.

A study in Japan was recently reported of 39 patients with early lung cancer, treated with Photofrin and light irradiation delivered by EDL through a flexible bronchoscope [122]. Cure rates were high for small (< 1 cm length) lesions, with a complete response in 32 of 40 lesions. Sutedja et al. [123] performed a pilot study of Photofrin PDT on 26 patients. The group with Stage I disease had a CR rate of ten of 11 patients. The patients with Stage III disease had little clinical benefit, showing either partial response or tumor progression. The four patients who died (within 6 months of PDT) had previously failed radiation therapy, Nd:YAG laser, and brachytherapy.

Okunaka et al. [124] had treated 145 lung cancer patients with PDT and reported the effectiveness of Photofrin PDT in 13 patients with

multiple primary bronchial carcinoma. Three patients had only early stage lesions and received no surgery additional to PDT, whereas ten patients required surgery for advanced lesions. Patient survival ranged 14 to 87 months, with seven alive at the time of report.

Shimatani et al. [125] treated seven patients, mostly with Stage I early lung cancer, with PDT by administering the Photofrin by bronchial arterial infusion (BAI). For this pilot study, the Photofrin dose was 0.7 mg/kg, about one-third of the usual dose employed. An EDL emitting 630 nm light was used at a dose of 100 J/cm^2, via fiberoptic bronchoscope at 72 h after BAI. Complete remission was achieved in five Stage I cases and a partial response achieved in two patients, which were a recurrence case and an advanced stage case.

Gastrointestinal cancer. This group includes esophageal, gastric, and colorectal cancer. Early stage esophageal lesions are treatable by surgery. Advanced disease involving varying degrees of esophageal obstruction carries a mortality of 10–20% after surgery, and many different palliative techniques have been introduced to relieve dysphagia. These include combinations of dilation, stents, Nd:YAG laser, BICAP thermal probes, and radiation therapy [126]. None of the available treatments offer long-term survival if esophageal cancer is advanced at the time of diagnosis, so early diagnosis is essential.

PDT appears promising for treating early or superficial esophageal tumors and as a palliative treatment for malignant dysphagia [127]. A Phase III trial for esophageal cancer includes patients with partially obstructing esophageal lesions. The patients are randomized to Photofrin PDT or Nd:YAG laser treatment. Patients with completely obstructive disease can receive Photofrin PDT as part of a Phase II single-arm protocol [118]. PDT is also being evaluated for the condition known as Barrett's esophagus, in which columnar epithelium replaces normal malpighian epithelium [128,129]. The incidence of carcinoma is 10% in these patients. Currently, two patients with Barrett's esophagus with early adenocarcinomas have been treated with Photofrin PDT [129]. Variation in response was noted because of insufficient light delivery to esophageal folds.

Overholt and colleagues [130] have developed a cylindrical esophageal balloon device for delivering circumferential light to the center of the lumen for PDT of esophageal cancer. The balloon is specifically intended to distend and flatten esophageal folds. Inside the balloon is a clear tube for holding a cylinder diffuser-tip fiber. Three isotropic probes on the outside of the balloon measure the delivered light dose to the esophageal mucosa. Uniform light irradiation was achieved, compared to use of the cylindrical diffuser without the balloon device.

In Japan, 80 patients with upper gastrointestinal tumors were treated with HPD and ADL light delivered endoscopically [131]. PDT was most effective for superficial esophageal cancer and poorly defined gastric cancer lesions. Okunaka et al. [132] treated 20 patients by PDT, six with early superficial esophageal carcinoma, and 14 had advanced invasive disease. PDT was performed through a biopsy channel of the gastroscope. Treatment was effective for early esophageal cancer (4/6 had complete remission), whereas advanced cancer patients experienced only improvement in dysphagia. McCaughan (133) reported the results of 40 patients receiving PDT as palliative treatment; 19 had adenocarcinomas, 19 squamous cell carcinomas, and two had melanoma lesions of the esophagus. The treatment goal was to improve swallowing in the patients, which proved to be of short-term benefit. Average survival time was 7.7 months for adenocarcinoma and 5.8 months for squamous cell carcinoma. In China, 142 patients with a variety of advanced gastrointestinal tumors were treated with HPD 48–72 h before ADL (630 nm light) treatment [134]. Fifteen patients showed CR (10.6%) and 53 showed PR (37.3%).

Gastric cancer normally presents in advanced form in most parts of the world and is associated with high mortality. Japan has implemented screening protocols involving endoscopic ultrasound and biopsy, with the result that early diagnoses are being made and the mortality rate has decreased [135]. Early gastric cancer is conventionally treated by surgery, and in Japan, patients have received PDT who refused surgery. Kato et al. [136] treated 19 patients (20 adenocarcinoma lesions) with Stages I–III gastric cancer, using HPD or Photofrin PDT. Red (630 nm) light supplied by an ADL or EDL was delivered through a fiber passed down the instrument channel of a gastrofiberscope. A CR was reported in 11 of the 19 patients (60%). Incomplete responses were thought to be due to inadequate light dosage, either because of the tumor's location or because of extensive or invasive growth into the muscular layer.

Colorectal cancer is treated by surgery as the

treatment of choice, but prognosis for recurrence depends on degree of spread outside the colon or rectum. By the time deep tumor invasion is present, treatment is intended to be palliative using Nd:YAG thermal ablation therapy to control hemorrhage or obstruction [137]. Barr et al. [138] reported the results of ten patients with inoperable colorectal disease treated with HPD PDT as an alternative to Nd:YAG laser therapy. The advantage of PDT over thermal ablation appeared to be preservation of the submucosal collagen layer. As a result, the colon retained mechanical strength, which removed the risk of perforation (the potential complication after Nd:YAG laser), and healing by rapid regeneration occurred. The conclusion of this study [138] was that a combination of Nd:YAG laser for tumor debulking and PDT for small or residual disease might produce optimal results.

Superficial bladder cancer. This cancer can present as papillary tumors or as carcinoma in situ (CIS). Papillary bladder cancer is conventionally treated by transurethral resection (TUR). The recurrence rate is high (ranging 40–70%) following TUR, and prophylactic intravesical chemotherapy has been found to significantly improve the long-term response [139]. PDT Phase III trials are underway for prophylaxis of recurrent papillary bladder cancer. After TUR of tumors, patients receive Photofrin (2 mg/kg) and low dose light (15 J/cm^2) to the whole bladder [118]. CIS is a high grade and aggressive manifestation of transitional cell carcinoma of the bladder, which previously indicated cystectomy [140]. However, intravesical BCG therapy now produces uniformly good responses, so that cystectomy is no longer the appropriate initial treatment [140]. A Phase II study for CIS is being performed in Europe and the United States of America in which PDT is an alternative to cystectomy. Patients receive Photofrin followed by whole bladder PDT, using the parameters described above for the Phase III (papillary bladder cancer) trial [118].

Irradiation of the whole bladder (or sometimes combined focal and whole bladder irradiation) is now the preferred procedure for PDT, because bladder cancer is often multifocal. The superficial tumors are often difficult to detect cytoscopically, so there is a risk of missing tumors with focal irradiation only [102]. Several methods are used for uniform irradiation of the whole bladder. Intralipid is a fat emulsion that acts as a light-scattering medium and makes it possible to use a flat cut fiber for laser treatment. Otherwise, many investigators use a spherical diffuser-tip fiber, which can emit light isotropically. Specially designed double balloon catheters can be used to position the tip. Unsoeld et al. [141] have reported on a new type of balloon coated with a light-scattering material, exhibiting ~90% reflectivity. It is inserted into the bladder, then filled with water so it unfolds spherically. Marijnissens group [142,143] developed a delivery system using a modified cystoscope to introduce a fiber with diffusing tip into the bladder and three nylon catheters that unfold in different directions along the bladder wall. Each catheter incorporates an isotropic light detector providing a measure of integrated light dose.

Nseyo et al. [144] described the development of an intravesical laser catheter (IVLC) delivery and monitoring system. The IVLC provides several advantages compared to simply positioning the light by cystoscopy and ultrasound. Mainly, it protects against nonuniform photoirradiation. The system automatically results in the tip being positioned within the center of the bladder. Inflation of the catheter's balloon transforms an asymmetrical bladder into a sphere of known diameter. A light sensor is incorporated in the balloon wall to monitor light fluence and dose and is computer controlled to adjust the total dose.

Nseyo reviewed results of PDT for papillary bladder cancer and reported that eradication rates depend on the tumor size [145]. Widespread micropapillary disease and tumors <2 cm diameter can be completely eradicated. When all patients were included in the assessment, single PDT treatment produced CR rates of 70–95%.

Jocham et al. [146] treated 20 patients with recurrent superficial CIS by whole bladder PDT. Cases that were resistant to intravesical BCG therapy and chemotherapy proved to be highly sensitive to this modality. Six of the 20 patients treated with PDT alone remained free of disease during a 5-year follow-up. The remainder of the patients received TUR and Nd:YAG laser therapy additional to PDT in order to achieve remission. Nseyo [145] reports the response rate of CIS treated by whole bladder PDT (total 37 patients) to be CR 88%, with an incidence of 25% recurrence during a 12–60-month follow-up. In patients undergoing PDT prophylactically, the recurrence rate was 31% with a median time of 18 months to recurrence.

Guo [147] reported on the treatment of 40 patients with superficial bladder tumors (104 lesions). Argon green light (514 nm) was chosen for

irradiation, even though tissue penetration is only around 1 mm [148]. Light was delivered locally to visible lesions either by surface or interstitial fibers. The whole bladder was subsequently irradiated with 2–3.5 J/cm^2 green light to reach small multifocal tumors. All patients showed CR initially, and seven of 40 patients had recurred during the reported follow-up period of 7–34 months.

Brain tumors. Surgical excision is the primary treatment for most brain tumors, although the success rate is dependent on the tumor type, degree of encapsulation, and location. Typically, the most malignant tumors, such as glioblastomas, are not encapsulated and postoperative radiation therapy is indicated [88]. Local recurrence of the tumor is the main reason for treatment failures. Median survival is <1 year from time of diagnosis [149]. Nd:YAG laser hyperthermia is also currently under evaluation for residual and recurrent tumors [150].

PDT has been used most often as a treatment to prevent recurrence of supratentorial high grade gliomas after surgical resection, but it is possible that PDT may be of value in other intracranial tumors such as low grade gliomas. Pineal gland and pituitary gland tumors may be treated with PDT as an adjuvant therapy, since complete excision is often difficult [88]. The use of photodynamic therapy in combination with stereotactic equipment is an exciting possibility for treating small, deep-seated unresectable gliomas [150]. A direct correlation has been measured between the grade of glioma and porphyrin level in the tumor. The levels were highest in glioblastoma multiforme (mean 5.9 μg HPD/g tumor wet weight) and lower for the intermediate grade anaplastic astrocytoma (2.4 μg/g) and low grade astrocytoma (1.6 μg/g). Uptake into normal brain tissue of HPD sensitized patients was 0.2 μg/g [151]. The blood-brain barrier is thought to play a role in attenuating the delivery of photosensitizer, so that some brain tumor cells will be spared. Intratumoral injection may be advantageous compared to intravenous administration of photosensitizer [152].

Light delivery systems have been developed for treating brain tumors by PDT. It is important to shield the laser tip and prevent local charring. A device for delivery of light to postresection tumor beds was developed by Muller and Wilson [153–155]. Over 50 patients with malignant supratentorial gliomas have received intraoperative PDT by this group. Patients were injected with porphyrin photosensitizer, and 18–24 h later a craniotomy with tumor resection was performed. The resultant cavity was photoirradiated through an inflatable balloon applicator filled with intralipid to scatter light. The device also comprised intrinsic light detection. Muller and Wilson [153] determined light penetration depth to be 2.9 mm depth in tumor and 1.5 mm depth in normal brain. It will be necessary to develop new light delivery devices for treating areas of brain to several cm depth. For 12 of the 50 patients, a complete immediate response to PDT was achieved. The median survival for this group was 17 months. In 33 cases, which were all primary malignancies, a partial response was noted and median survival was 6.5 months.

Perria et al. [156] treated eight recurrent brain tumor patients with intraoperative PDT who had all previously undergone surgical resection and radiation therapy. HPD was given 24 h before surgery and the residual tumor bed exposed to red light. Survival in a few patients appeared to be lengthened, although all patients ultimately had recurrence. Kaye et al. [157] reported a series treating 45 patients, consisting of 37 glioblastomas, seven anaplastic astrocytomas, and one metastasic lung lesion. A laser dose escalation study was performed, using light generated by an ADL for 15 patients and GVL for 30 patients. Results were comparable with both lasers. The need for high light doses in the treatment of brain tumors by PDT has been recognized, as well as the use of combined intracavitary and interstitial photoillumination [149].

Gynecological cancer. Current treatment options for superficial noninvasive gynecological cancer include surgery, cryotherapy, Nd:YAG laser, and CO_2 laser vaporization [158]. The majority of gynecology patients treated with PDT have had cervix or vaginal carcinomas. A few patients with local endometrial and ovarian carcinomas have also been treated [118]. Most studies have comprised only a small number of gynecological cancer patients [118]. A series of 21 patients with recurrent tumors was reported by Lele et al. [159]. Endoscopic or surface delivery of light was employed. All patients experienced significant discomfort at the treatment site. CR was achieved in nine patients and PR was obtained in two patients. Optimization of PDT for gynecological lesions is required, particularly in regard to light delivery.

Head and neck cancer. Head and neck malignancies are treated at present by surgery with radiation therapy and/or chemotherapy. Lo-

cal or regional recurrence of tumor is common, and further surgery is usually carried out [160]. Initially, only patients with advanced disease (Stages III and IV) were treated with PDT [161]. The treatments, intended to be palliative, met with limited success. Results of PDT for superficial and early tumors of the head and neck are considerably more promising, often saving patients from additional surgery [162]. PDT also appears promising as an adjuvant intraoperative treatment of recurrent head and neck carcinomas [163]. Generalization of the laser procedure is difficult because of the varying geometries of these cancers. Forward surface photoirradiation or cylinder-diffusing delivery systems inserted through a laryngoscope are usually used.

A preliminary investigation of PDT efficacy was carried out in 12 patients with squamous cell carcinomas localized in the nasopharynx, palate and uvula, larynx and retromolar trigone [164]. One patient had no response, and the remainder showed a CR (50%) or PR (50%). Feyh et al. [165,166] reported a study of 94 patients with various superficial head and neck tumors (disease status ranged CIS-T2. HPD was injected 48 hours before 630 nm light treatment. A CR of 95% was confirmed histologically 2 months after PDT. Five patients relapsed during follow-up (maximum 4.5 years). Biel [162] reported on the PDT treatment of 49 patients. All 26 patients with CIS and T1 laryngeal or nasopharyngeal carcinomas obtained a complete response. Three patients recurred, whereas 23 patients remained disease-free for up to 32 months. Eight patients with T2 and T3 carcinomas obtained CR or PR, but all cases recurred locally. Treatment of advanced cancer in four patients resulted in regrowth occurring within 1–3 months. Wenig et al. [167] evaluated HPD PDT for squamous cell carcinoma of the head and neck in 26 patients. The CR rate was 76% during the 48-month follow-up.

A small study examined PDT as an aduvant treatment to surgery in comparison with radical surgery alone [163]. Four patients with recurrent infiltrating carcinomas of the head and neck received Photofrin 48 hours before total surgical excision and laser irradiation (50 J/cm^2) of the resection bed. Follow-up was 6–8 months, during which all patients remained free of disease. Therefore, the results of intraoperative PDT appear promising, especially since Stages III and IV infiltrating carcinomas have a high rate of recurrence (>50%) after surgical and radiotherapy treatments.

Ocular cancer. In adults, the commonest ocular malignancy is choroidal melanoma, with prognosis depending on histological type and tumor size at diagnosis. Enucleation is the primary treatment for large lesions, although ocular brachytherapy and local surgical resection can be tried in an attempt to preserve the eye [168,169]. Retinoblastoma is the most common eye tumor in childhood. A variety of treatments are used, particularly in bilateral cases of retinoblastoma in an attempt to salvage the vision in at least one eye. Options apart from enucleation include external beam radiation, episcleral brachytherapy, and chemotherapy with or without laser hyperthermia [170].

The accessibility of ocular tumors and the optical properties of the eye are compatible for PDT. Preclinical and clinical reports evaluated PDT using transpupillary and transscleral delivery of laser light [171,172]. The transpupillary route produces direct photosensitization of the tumor mass, whereas transscleral delivery is intended to destroy the choroidal blood supply to choroidal melanomas.

Several groups have reported their preliminary clinical results for small numbers of patients. The largest series included 24 patients with choroidal, iris, or ciliary body melanomas treated with HPD PDT [171]. Red (630 nm) light was delivered both transpupillary and transsclerally. All small to medium-size tumors (<1,000 mm^3) tumors responded initially, and some complete responses were achieved during a 7 year follow-up. Larger tumors recurred and required enucleation. Murphree et al. [173] treated seven choroidal melanoma patients, one iris melanoma, one ciliary body melanoma, and six retinoblastoma patients with ocular PDT. Complete responses were obtained in two amelanotic melanomas, whereas responses in pigmented choroidal melanomas were minimal due to attenuation of the light. Retinoblastoma tumors without evidence of vitreous seeding initially responded, but were not cured long term. Avascular tumor seeds in the vitreous did not respond to PDT, presumably because they contained insufficient HPD and/or had insufficient oxygen available for the photodynamic process.

Cutaneous and subcutaneous cancer. Conventional treatments for cutaneous and subcutaneous malignancies include surgery, radiation, and chemotherapy. Satisfactory cure rates can be achieved with current modalities, but alternative modalities are necessary for extensive

or multiple lesions, such as superficial basal cell carcinomas (BCC) and Bowen's disease [174]. The results of widespread surgical excision and irradiation can be cosmetically unacceptable for a patient.

McCaughan et al. [175] reported on 27 patients with cutaneous and subcutaneous malignancies (a total of 248 lesions) treated by PDT. Diagnoses included BCC, squamous cell carcinoma, metastatic breast cancer, malignant melanoma, liposarcoma, and Bowen's disease. The total CR observed during a 1-year follow-up was 48%. Carruth [161] also found this modality to be effective against Bowen's disease and multiple BCC in a pilot study. The initial clinical response of all patients was excellent, but recurrence developed in BCC lesions. Wilson et al. [176] carried out a prospective study in 37 patients to determine the effectiveness of Photofrin PDT for primary or recurrent basal cell tumors (151 tumors). A CR rate of 88% was achieved with one treatment session. Jones et al. [177] treated six patients with Bowen's disease, with Photofrin and red light, achieving 100% CR after 12 months of follow-up. Lowdell et al. [178] reported their results of treating nine patients with PDT. Fifty cutaneous or subcutaneous tumors, with volumes of up to 60 cm^3, were treated with interstitial irradiation. Another 22 tumors in these patients received surface irradiation. The total CR rate in this study was 81%. Khan et al. [179] treated a series of 37 patients with cutaneous metastatic breast carcinoma. Effective PDT was achieved using a reduced Photofrin dose of 0.75 mg/kg with the light dose increased to 180 J/cm^2. The conclusions from skin malignancy studies are that the size of the lesions is an important determinant of response, as well as the observation that PDT can produce superior healing of normal tissue without scarring.

Kaposi's sarcoma. HIV-positive patients are susceptible to various types of malignancy, but AIDS-related Kaposi's sarcoma (KS) is the most common and is an aggressive form of sarcoma. Chemotherapy or immunotherapy, radiotherapy, and surgical excision have been used with limited success [180]. KS is a multicentric tumor of vascular endothelial cell origin, which suggests PDT will be effective when mediated by photosensitizers that damage endothelium. Light delivery is either by surface irradiation for diffuse superficial lesions or interstitial for nodular lesions. Schweitzer [180] has treated eight KS patients with Photofrin PDT. Treatment was intended primarily to control large lesions in the oral cavity, either alone or after debulking surgery. Short-term palliation was achieved and the lesions could be retreated. Biel [162] treated two patients with extensive KS of the hard and soft palate, with at least two sessions of PDT. Response was variable; the flat lesions responded, but nodular lesions showed no response.

Comparison of Photofrin PDT and Laser Thermal Ablation as Single Treatments

This section compares PDT and laser thermal ablation therapy (using the Nd:YAG laser) for treating malignant lesions. Randomized clinical trials are being carried out that make this comparison.

McCaughan [181] compared PDT and Nd:YAG laser treatments for endobronchial and esophageal malignancies. Laser treatment times during bronchoscopy were comparable, although Nd:YAG laser reduced the size of obstruction more at the end of a treatment. After clean-up bronchoscopy following PDT, the degree of obstruction was similar. A distinct difference in tissue reaction was observed for the two modalities several days posttreatment. PDT created a fibrinous plug that could be lifted off the bronchus in large pieces. The YAG laser typically produced a burn with coagulated and charred tissue, which was more difficult to remove because it fragmented. PDT was technically easier to perform than thermal laser resection and coagulation, since it was associated with lower risks of bronchial or esophageal perforation. In the case of obstructive emergencies, the disadvantage of PDT that the photosensitizer needs to be administered 1 or 2 days prior was overcome by same day injection and laser. Nd:YAG therapy was considered more effective for debulking large or bleeding lesions, whereas PDT was superior for treating small or residual tumor, producing necrosis cleanly to the bronchus wall. Treatment of patients with thermal ablation followed by PDT a few weeks later utilizes the advantages of both techniques.

In Norway, the Nd:YAG laser is used effectively to produce cures in selected cases of bladder cancer (CIS and recurrent transitional cell carcinoma), as an alternative to TUR. Nseyo [182] discussed Nd:YAG therapy and PDT for treatment of superficial bladder cancer. Thermal ablation produced thick tumor necrosis to a depth of 5–6 mm and sealed lymphatic drainage, which may prevent tumor dispersion. However, energy-depen-

dent injury to contigous organs such as the bowel were possible following laser ablation. The YAG laser is also occasionally used for palliation of locally invasive bladder cancer, when curative cystectomy was contraindicated. PDT using whole bladder laser irradiation was less penetrating than YAG and was suitable for CIS and recurrent superficial lesions following TUR, producing 90–98% response rates. It represents a useful alternative modality for superficial disease.

Intracavitary PDT

Laser treatment of malignancies in the peritoneal and pleural cavity via intraoperative PDT is currently being examined. A Phase I study was initiated using intracavitary PDT for peritoneal carcinomatosis [183]. Patients received DHE 48 hours before laparotomy and debulking surgery, then were treated with light to intra-abdominal surfaces using 0.2–0.5% intralipid to enhance light diffusion. Photodiodes were sewn into the peritoneal cavity for in situ dosimetry. DeLaney et al. [184] reported the results of 54 patients treated as part of the Phase I study. Initially, 630 nm light at 2.8–3 J/cm^2 was used, but small bowel edema occurred with perforation in three cases. Light dose escalation was achieved by using green (514 nm) light, up to 3.75 J/cm^2. No small bowel complications occurred.

Intraoperative PDT was extended to treatment of pleural malignancies, such as mesothelioma or isolated pleural metastases [183]. Similarly, laser light was delivered to the thoracic cavity and photodiodes were sewn into the chest area. The postoperative course in patients was unchanged, and the efficacy of PDT as an intraoperative adjuvant therapy awaits results of future prospective clinical trials.

Sindelar et al. [185] also report on the use of intra-abdominal PDT for disseminated malignant disease, in 23 patients. Following resection, 630 nm light was delivered to peritoneal surfaces at escalating doses ranging from 0.2 to 3 J/cm^2. Viscera were anatomically isolated for laser exposure. Six patients were disease-free after 18 months. Five patients had significant treatment complications.

These preliminary studies suggest that intracavitary PDT will be evaluated in Phase II and III studies to determine efficacy for these types of tumors that have a typically high risk of recurrence. The goal is to convert surgical partial responses to complete responses. Regional toxicity may be a potential concern. Several experimental studies have evaluated the thresholds for damage and toxicity to abdominal organs [186]. Dose ranges were defined in each study that would not result in normal tissue necrosis. Pelton et al. [187] exposed large pleural surfaces to PDT and produced a spectrum of tissue specific injury in intrathoracic organs. Therefore, the risk of complications from locoregional toxicity after intracavitary PDT is currently unknown.

Bone Marrow Purging

Autologous and allogeneic bone marrow transplantation are used to treat leukemias and selected solid tumors. Autologous transplantation offers several advantages, notably avoiding the risk of graft rejection, viral infections, and lymphoproliferative disorders from graft manipulation. Unfortunately, relapse rates tend to be higher in autologous marrow grafts [188].

PDT is one of the newer techniques for extracorporeal bone marrow purging, and several photosensitizers have been proposed for photodynamic treatment of remission marrow, including DHE, BPD, ClAlPc, and merocyanine 540 (MC 540). Bone marrow grafts consist of free-flowing single cells in suspension, which are amenable to uniform exposures of photosensitizer and light. A significant advantage of this technique is that the drug can be removed before reinfusion of the treated cells into the patient, thus avoiding systemic photosensitization. MC 540 has been widely tested in preclinical models. The dye preferentially binds to electrically excitable cells, leukemia/lymphoma cells, and some virus transformed cells [188]. Under conditions that preserve 50% of human pluripotent hematopoietic progenitor cells, PDT can reduce the concentration of clonogenic promyelocytic leukemia cells and CML by up to 8 log [189]. Purging of non-Hodgkin's lymphoma (NHL) from autologous marrow grafts has been specifically explored [190]. MC 540 PDT produced 4–5 log eradication in vitro of patient-derived NHL, at doses which preserved ~50% of normal hematopoietic progenitor cells. MC 540 is the first agent to be evaluated in a Phase I clinical trial, for purging of leukemia and lymphoma cells [191]. The clinical application of MC 540 PDT found that several-fold higher doses were tolerated than used in preclinical models.

In addition, T- and B-cell immunity were found to be suppressed by MC 540 sensitized photoirradiation [192]. As a result, treatment may affect immune reconstitution in autologous marrow graft recipients. In allogeneic grafts, these

immunomodulatory effects could reduce graft rejection in the situation of partially mismatched marrow transplants.

Clinical and Preclinical Studies of Second-Generation Photosensitizers

Benzoporphyrin derivative. BPD is synthesized from protoporphyrin and has an absorption peak at 690 nm four times greater than Photofrin's absorption at 630 nm. The mono-acid form has more photodynamic potency than di-acids [193], and the mono-acid was used for all studies described in this review. BPD uses lipoproteins for localization in vivo and particularly associates with tumor cell membranes [194]. Like all sensitizers, BPD does not have specific affinity for tumor tissue, reaching higher concentrations in the liver, spleen, and kidney. BPD has the purported advantage, in addition to its 690 nm absorption, of a favorable distribution between tumors and normal skin within a few hours of injection [195]. This property is expected to result in less skin photosensitivity as a side effect: BPD-MA is showing promise in Phase I/II clinical trials for skin tumors. Similar selectivity in BPD uptake by tumor cell lines (5–10-fold increase) occurs in activated T lymphocytes, compared to normal splenic lymphocytes [196]. Since activated T cells are responsible for the symptoms of most autoimmune diseases, preclinical studies are being carried out as a possible treatment for autoimmune conditions such as systemic lupus erythematosus [196]. BPD is undergoing preclinical testing for its ability to photoinactivate retroviruses in cells and blood [197], and also to treat atherosclerosis [196].

Mono-aspartyl chlorin e6. NPe6 is a chlorin photosensitizer with properties of very short term photosensitivity and a high extinction coefficient at 664 nm. Interestingly, preclinical studies found that PDT-mediated tumor cures correlated with the plasma concentrations of NPe6 rather than the tumor tissue levels of photosensitizer [198]. Maximal tumor response was achieved by irradiating tumors at 4–6 hours after sensitizer administration. NPe6 has been examined in a preliminary clinical study to patients with superficial malignancies [199]. Patients had diagnoses of primary or metastatic skin, oro- and nasopharynx cancer. Drug was injected 4–8 hours prior to irradiation with 664 nm light. Overall, CR was achieved in 11 of 20 tumors treated, four were PR, and the remainder were no responses. The maximum tumor necrosis was measured as 8 mm, whereas normal tissue had 1 mm necrosis or less, indicating relative tumor selectivity by NPe6 PDT at the treatment times used. This was in spite of high NPe6 levels in the circulation and normal skin during treatment. Drug elimination was complete by 4 weeks after drug administration in all patients.

Meta-tetra(hydroxyphenyl)chlorin. mTHPC was synthesized and evaluated in preclinical studies by Berenbaum [200]. In rodent models, mTHPC was found to have both improved tumor tissue selectivity and antitumor activity compared to DHE. It has an absorption peak at 652 nm. Initial clinical results with mTHPC were published by Ris et al. [201] following treatment of patients with chest malignancies. Initially, two patients received an injection of mTHPC and 652 nm laser irradiation. Parameters were 0.3 mg/kg mTHPC, 48 h prior to light exposure of 10 J/cm^2. Biopsy samples showed tumor infarction 10 mm deep due to tumor vessel thrombosis, and the concentration of chlorin sensitizer was 14 times higher in mesothelioma tumor tissue than normal tissues. A further eight patients with diffuse malignant mesothelioma received intraoperative PDT to the thoracic cavity following unilateral pleurectomy and lobectomy [201,202]. The patients developed recurrences, although mostly in untreated areas. The conclusions drawn from the intraoperative treatments were that the procedure is feasible, but significant morbidity can occur when large areas are treated. Optimization of the therapeutic ratio is essential in order to prevent extensive damage to normal tissues during intracavitary mTHPC PDT.

Tin etiopurpurin. SnET2 is a metallochlorin with potent photosensitizing properties [203, 204]. It is hydrophobic and requires solubilization in a suitable drug delivery system, such as a lipid emulsion, for in vivo use. SnET2 has an absorption peak at 660 nm, which is used for photodynamic treatment. It is purported to produce significantly reduced photosensitization of normal tissue compared with DHE at the therapeutic dose [205]. Tissue distribution properties and clearance kinetics are comparable for both drugs, and similar drug injection to laser intervals can be employed for treatment [205]. Preclinical results are sufficiently encouraging that SnET2 is commencing Phase I/II clinical trials in the United States.

Amino-levulinic acid. Administration of exogenous ALA enhances the biosynthesis of endogenous PpIX for production of heme in certain types of cells and tissues [206]. The subsequent

conversion of PpIX to heme is a relatively slow step, resulting in transient accumulation of protoporphyrin to sufficient levels that it can act as a photosensitizer.

Preclinical studies have been carried out to investigate ALA administration by topical application, intradermal injection, subcutaneous injection, intraperitoneal injection, and orally [207]. Systemic routes produce generalized photosensitivity, but are required for tumors that are too thick to be reached by local administration. Loh et al. [208] found comparable kinetics of PpIX in animals after intravenous and oral ALA administration. PpIX predominantly accumulated in mucosa of skin, colon, and bladder, with little in the submucosa and smooth muscle layers. Subsequent light treatment resulted in mucosal ablation only. Three patients were administered oral ALA, and biopsy samples demonstrated preferential PpIX accumulation after 4–6 h [208]. Following topical application of ALA (10% oimtment) to BCC lesions, fluorescence measurements showed PpIX accumulation only in normal skin after 4 hours. A 12-hour interval was required in order for tumor cells situated in lower dermis to become maximally fluorescent [209].

Several clinical studies have been reported evaluating topical ALA mediated PDT for treatment of cutaneous malignancies [207,210–213]. Topical solution (20%) of ALA is applied before same-day laser irradiation with 630 nm light. Bowen's disease lesions and BCC lesions show the highest response. One clinical trial showed a CR rate of 90% and PR rate of 7.5% in the first 80 BCC patients treated [207]. Similarly, Bowen's disease lesions obtained a CR of 89% at 18 months follow-up [211]. Warloe et al. [211] reported on 11 patients with 94 lesions of BCC, treated with ALA PDT. At 3 months post-PDT, 90 lesions (96%) were evaluated to be CR, although 13% had required more than a single PDT treatment. Lesions thicker than 3 mm may achieve a 40–50% CR [216,218]. Metastatic lesions (adenocarcinoma and melanoma) and noduloulcerative BCC lesions have shown consistently poor resposes [210,213a]. Superior cosmetic results appear to be obtained using ALA PDT in the studies.

NONONCOLOGIC APPLICATIONS OF PHOTODYNAMIC THERAPY

PDT of Viral Diseases

The first photodynamic studies on viruses were on bacteriophage, where it was found that penetration of the sensitizing dye was a variable factor [214]. A number of animal viruses, including adenoviruses and vaccinia viruses, were shown to be inactivated by PDT. Resistant viruses could be made sensitive to PDT by incubating with dye under conditions that increased viral coat permeability [215]. The earliest patient treatments were for herpes simplex viral infections of the skin, using neutral red dye and white light [216]. The efficacy of antiviral PDT is still undergoing preclinical investigation, using various photosensitizers and light delivery systems.

Papillomavirus. PDT has been proposed as a possible treatment for papillomas of the larynx. Laryngeal papillomavirus lesions are initially benign but can become serious and potentially life-threatening. The lesions are surgically removed, but typically the disease is marked by multiple recurrences and a prolonged clinical course [217]. Disease occurs with equal incidence in children and adults.

Abramson et al. [218] treated 33 patients with laryngeal papillomatosis using DHE PDT. The severest cases responded without recurrence during follow-up. Feyh et al. [165] treated 21 patients with recurrent laryngeal papillomatosis as part of a pilot study of HPD PDT for malignant superficial cancers of the head and neck. The study showed a CR rate of 95% over 4 years of follow-up. Although these results appear promising, PDT cannot remove latent infection of papillomavirus in normal tissue. The risk/benefit ratio of PDT treatment for the more frequent problem of cutaneous and genital warts remains undetermined.

HIV and blood-borne viruses. There is an accumulating amount of data that PDT can be used to effectively eliminate pathogenic enveloped viruses from infected cells, cell-free suspensions, and whole blood (219–222). Susceptible viruses include human immunodeficiency virus type I (HIV-1), herpes simplex virus type I/II (HSV-1,HSV-2) type I, human cytomegalovirus (CMV), measles, and simian virus (SIV).

The photosensitizers being evaluated for PDT-mediated viral inactivation include DHE, BPD, aluminium phthalocyanine, and merocyanine 540 (MC 540). Photoinactivation is thought to occur by oxidative modification of the lipid and protein components of the viral envelope. The mechanism of MC 540 antiviral activity has been most studied [223,224]. The available data suggest that MC 540 PDT damage to the virus envelope, in the form of extensive cross-linking, interferes with

early events in the infectious process, the ability of the virus to adhere and to penetrate the cell. Since these photosensitizers do not target the nucleic acid of the virus, they are ineffective against non-enveloped viruses, such as poliovirus type I and human adenovirus-2 [219]. One advantage of dyes that do not interact with viral DNA is that they do not have inherent mitogenic properties.

PDT is being evaluated as a potential blood transfusion sterilizing system against pathogenic organisms. Obviously, the formed elements and noncellular components of blood must not be functionally damaged by the treatment. Some loss of activity of coagulation proteins such as factor VIII and von Willebrand factor is acceptable. The expense and complexity of implementing PDT as a sterilization system in a blood bank environment are also important factors that have to be considered [219]. Matthews et al. [220] did not detect damage to erythrocytes, complement factors, and immunoglobulins directly after DHE and BPD mediated PDT of blood, cells, and viral suspensions. Sieber et al. [221] demonstrated MC 540 PDT inactivation of a wide variety of viruses at concentrations that caused little photosensitivity of red cells, factor VIII, and von Willebrand factor. Naturally infected blood (with HIV-1) and spiked human blood have been tested after BPD PDT [225]. Free virus and infected (activated) leukocytes were effectively treated, whereas red cells and uninfected leukocytes were spared.

In another study by North et al., the red cells showed potassium leakage and IgG binding, indicating some damage occurred from photodynamic treatment [222]. This observation together with incomplete free HIV kill in their model system suggests that commercial sterilization of blood and blood products might not be feasible. However, the preferential sensitivity of activated cells (like leukocytes) is considered a real advantage since HIV replicates only in activated CD4 positive T cells [222]. Studies that exploit this result are planned to evaluate PDT as a treatment to reduce the HIV burden in patients. Extracorporeal treatment of blood or leukocytes in HIV-infected individuals seems to stabilize or improve immune function, perhaps by a stimulatory effect of the inactivated virus or by modulation of activated leukocytes. PDT would provide a beneficial treatment modality in this respect.

PDT of Atherosclerosis

Atherosclerotic vascular disease is the leading cause of death in the world [226]. The possibility of treating atherosclerosis with PDT is based on the observation that atherosclerotic plaques take up higher concentrations of porphyrin than normal vessel wall. Preclinical studies showed that DHE, NPe6, and TPPS were found mainly in the interstitial space of plaques, not intracellularly [226]. The drugs were absent in the normal vessel wall and the wall underlying the plaques, which suggests these structures will not be damaged. BPD uptake was measured in atherosclerotic human arteries in vitro and in miniswine arteries in vivo, and again showed potential for treating atherosclerosis [227].

Vincent et al. [228] treated atherosclerotic plaques in miniswine with Photofrin PDT and 630 nm light, using a circumferential diffusing fiber tip. At 2 weeks post-PDT, angiography showed an average reduction in stenosis in 6/8 vessels from 71% to 19%. Interestingly, locally applied photosensitizer through a porous balloon catheter showed very high concentrations in the intima region in animals [229]. The advantage of local administration is that PDT would be feasible immediately after angioplasty and without adverse systemic effects.

Arterial intimal hyperplasia (IH) is the specific condition of restenosis in arteries and veins that were earlier treated for stenosis by transluminal angioplasty or bypass graft surgery. At present, no treatment exists for IH [230]. Smooth muscle cell proliferation in the intima, stimulated by platelet adhesion, is involved in the development of IH. It is possible that it might eventually be treatable by PDT [230,231]. Choroaluminium phthalocyanine PDT was evaluated for its ability to obliterate the IH response in a carotid artery model in the rat. The sensitizer was preferentially retained in the artery with induced IH. Circumferential homogeneous light was then applied to the whole artery. In contrast to untreated arteries, PDT-treated arteries showed a marked decrease in smooth muscle cell growth, as well as normal elastic luminae. Studies are required to determine if the positive response is maintained long term [231]. Interestingly, one study found a significant growth suppressive effect from DHE alone (in the absence of light) on smooth muscle cells from atherosclerotic primary stenosing and restenosing lesions, although the mechanism is unknown [232].

PDT of Skin Disorders

Psoriasis. Psoriasis is a common dermatological disorder in which the epidermal cells over-

proliferate, resulting in a clinical picture ranging from localized scaling plaques to generalized exfoliation of the skin. Treatment by PUVA phototherapy is an effective established method of controlling the increased cell proliferation. PUVA treatment comprises application of psoralen compounds (either topical or systemic 8-methoxypsoralen) to produce a photoadditive effect with UVA light [233].

Tin protoporphyrin (SnPP) photodynamically activated by UVA light has been proposed for treatment of psoriasis [234]. Repeated doses of UVA can be given for several weeks following a single injection. The photosensitivity of SnPP was investigated in 31 patients. Thresholds for UVA and visible light were lower after SnPP administration, but the UVB threshold was unchanged by this sensitizer. Mild erythema and mild conjunctivitis were experienced lasting several weeks to 3 months.

The first reported treatment with hematoporphyrin and light for psoriasis vulgaris was in 1937 [235]. Since then, there have been a few case reports using either systemic or topically applied photosensitizer. Berns et al. [236] treated one patient with HPD PDT, reporting that the psoriatic skin partially cleared. Treatment of 17 patients with palmoplantar psoriasis was evaluated by Pres et al. [237] using topical HPD ointment application and white light irradiation. All lesions responded, either significantly or totally resolving. In a recent pilot study, three patients with chronic psoriasis were treated every other day with PDT using topical 10% ALA [238]. No significant adverse effects occurred, and the lesions cleared with a similar time course as patients treated with dithranol. PDT using topical photosensitizer appears to be a beneficial psoriasis treatment, applicable to treat large surface areas.

Portwine stain. Portwine stain (PWS) is a congenital vascular lesion consisting of an abnormal set of capillaries in the upper dermis with a normal overlying epidermis. It most commonly occurs on the face and neck region. Treatment of PWS in the past included an array of modalities, such as skin grafting, ionizing radiation, and cryosurgery, all of which caused cosmetic scarring [239]. The introduction of the argon laser represented a major advance in PWS treatment. The blue-green lines of the argon laser correspond to hemoglobin absorption. The light is converted to thermal energy in the dilated ectatic capillaries and produces thrombosis in these vessels. Unfortunately, the epidermis receives some irreversible damage, since melanin and collagen absorb light. Use of longer wavelengths, such as 577 nm, has been shown to be preferable and leave less scarring. The extinction coefficient of oxyhemoglobin is higher than at 514 nm, whereas melanin absorption is minimized.

It may be possible to obtain selectivity using a photosensitizer and appropriate wavelength light, as shown in a chicken comb model by Orenstein et al. [240]. They used time intervals of 1–4 hours between Photofrin and blue (405 nm) light in order to confine damage to the vascular compartment. Fluorescence of HPD, indicating localization, was seen in a facial portwine stain by Keller et al. [241] in a patient who was being treated with PDT for bladder cancer. There do not seem to have been any patient series carried out of PDT treatment for benign vascular dermal lesions.

SUMMARY

After decades of basic and clinical research, PDT is on the verge of becoming an established cancer treatment modality. Its role will emerge when current Phase III clinical trials of Photofrin-mediated PDT are completed and treatment is in practice. The first product license approvals have been granted (outside the United States) for treatment of endobronchial, esophageal cancer, and superficial bladder cancers. Meanwhile, intracavitary PDT is still at the preliminary stages, but so far it appears promising. Certainly, some malignant diseases are more suitable than others with regard to whether complete eradication is possible. Very bulky lesions and tumors inaccessible to light irradiation remain untreatable by PDT. The efficacy and safety of PDT determined by clinical trial are not the only factors determining its future success, but also how the existing treatments for a disease compare. Development of resistance to PDT has not been noted in any patient tumors, which is a distinct advantage over some other anticancer modalities. Also, long-term morbidity does not arise to restrict the number of repeat treatments.

PDT is now being evaluated for wider applications, outside malignant solid tumor treatment. At the beginning of the century, photochemotherapy was realized to be potentially useful for a variety of indications, when photosensitization was first being observed in enzymes, viruses, cells, animals, and plants. Nononcologic applications of PDT are mostly at the preclinical stage and in-

clude viral inactivation in blood, modulation of immune function in autoimmune diseases, reduction in atherosclerosis lesions, and treatment of benign skin disorders. It is not possible to say at present which of these diseases or conditions will benefit most from PDT.

Development of second-generation photosensitizers is continuing, and dyes have already been designed with improved photodynamic properties. The side effect of skin photosensitivity can be diminished by dyes that absorb only in the far-red spectrum. Nonsystemic administration of drug or targeting techniques may also eliminate photosensitivity side effects. Classes of sensitizers that have been evaluated photochemically and biologically include porphyrins, chlorins, purpurins, and phthalocyanines. The most promising examples are being developed commercially. The technical development of user-friendly light sources, whether laser or nonlaser, is as important to the clinical applications of PDT as the choice of photosensitizer. Diode lasers generating sufficient power in the far-red visible region are only just becoming available for clinical use. In addition, specialized laser delivery systems continue to be developed, with respect to the specific site being treated. The methodology and technology used for photodynamic treatment of patients can be expected to change significantly for many years ahead. PDT is truly a dynamic process.

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Photodynamic Therapy Using Lu-Tex Induces Apoptosis In Vitro, and Its Effect Is Potentiated by Angiostatin in Retinal Capillary Endothelial Cells

Reem Z. Renno,[1] *Francois C. Delori,*[2] *Robin A. Holzer,*[1] *Evangelos S. Gragoudas,*[1] *and Joan W. Miller*[1]

Purpose. To examine the effect of combining angiostatin with photodynamic therapy (PDT) using Lutetium Texaphyrin (Lu-Tex; Alcon, Fort Worth, TX) as a photosensitizer in bovine retinal capillary endothelial (BRCE) and retinal pigment epithelial (RPE) cells and to determine the mode of PDT-induced cell death in these cell lines.

Methods. Cultured BRCE and RPE cells were incubated with angiostatin (500 ng/ml) for 18 hours and subjected to Lu-Tex/PDT, using treatment parameters previously optimized (3 μg/ml Lu-Tex for 30 minutes followed by timed irradiation at 732 nm). Cellular survival was assessed after a 1-week cellular proliferation. Data were analyzed using Student's *t*-test. Caspase 3 activity was monitored in cells after PDT using a fluorogenic substrate, (Asp-Glu-Val-Asp) AFC (7-amino-4-trifluoromethyl coumarin) [DEVD-AFC], of caspase 3. After PDT, expression of Bcl-2, Bcl-x_L, Bax, and Bak was also examined in cell lysates by Western blot analysis.

Results. A synergistic cytotoxic effect of angiostatin and Lu-Tex/PDT was observed in BRCE cells at all fluences used (5, 10, and 20 J/cm^2; $P \leq 0.05$). These findings applied only if angiostatin was delivered before PDT. No such interactive killing effect was observed in RPE cells. Caspase 3 activity was elevated within 10 minutes of PDT in BRCE and RPE cells and was fluence dependent. Differential modulation of Bcl-2 family members was observed after PDT in BRCE and RPE cells.

Conclusions. The combination of angiostatin and Lu-Tex/PDT potentiates the cytotoxic effect of Lu-Tex/PDT on BRCE but not on RPE cells. This may provide a strategy to increase the selectivity of PDT in damaging capillary endothelial cells with less damage to RPE cells. Lu-Tex/PDT induces rapid caspase-dependent apoptosis in BRCE and RPE cells. Furthermore, Lu-Tex/PDT induces apoptosis through selective modulation of members of the Bcl-2 family and differs between BRCE and RPE cells. (*Invest Ophthalmol Vis Sci.* 2000;41:3963–3971)

Age-related macular degeneration (AMD) is the leading cause of severe vision loss in people aged more than 65 years in Western countries.[1–3] Choroidal neovascularization (CNV) occurs in 15% of patients with AMD but accounts for 80% of severe vision loss due to AMD.[4,5] Photodynamic therapy (PDT) is showing promising results as a new modality for CNV.[6–9]

PDT involves the systemic administration of a photosensitizer dye that accumulates in proliferating tissues such as tumors and newly formed vessels. It is followed by irradiation of the target tissue with low-intensity, nonthermal light at a wavelength corresponding to the absorption peak of the dye.[10] Excitation of the dye leads to the formation of singlet oxygen and free radicals—better known as reactive oxygen species (ROS)—causing photochemical damage to the target tissue.[11]

Preclinical studies using PDT for the treatment of CNV have demonstrated that, with the proper treatment parameters of photosensitizer dose, laser light dose, and timing of irradiation, relative selective damage to experimental CNV can be achieved, sparing retinal vessels and large choroidal vessels and with minimal changes in the neurosensory retina.[12–15] However, in clinical studies, fluorescein leakage appeared in at least a portion of the CNV by 1 to 3 months of treatment, and increasing photosensitizer or light doses did not prevent the recurrence. This could also lead to undesirable nonselective damage to retinal vessels.[6] Several multicenter phase 3 trials are under way to study repeated PDT, applied every 3 months. The interim data look promising, showing decreased rates of moderate vision loss.[8] The necessity for repeated PDT can nevertheless be expected to lead to cumulative damage to the retinal pigment epithelium (RPE) and choriocapillaris, which may lead to progressive treatment-related vision loss.

Angiostatin, a proteolytic fragment of plasminogen that was first isolated from the serum and urine of tumor-bearing

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The Massachusetts Eye and Ear Infirmary is an owner of a patent covering the use of verteporfin. Should the Massachusetts Eye and Ear Infirmary receive royalties or other financial remuneration related to that patent, JWM and ESG would receive a share of same in accordance with the Massachusetts Eye and Ear Infirmary's institutional Patent Policy and Procedures, which include royalty-sharing provisions.

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mice, inhibits angiogenesis.[16,17] In vitro and in vivo studies have shown that radiation and angiostatin have combined cytotoxic effects on endothelial cells, and the combination of those two components has produced no increased toxicity to normal tissue.[18,19] These results provide support for further investigation of the effect of combining photodynamic therapy with angiostatin to improve CNV closure without damaging normal tissues. We tested whether angiostatin potentiates PDT-induced bovine retinal capillary endothelial (BRCE) cell damage, by inhibiting proliferation or by other means, without affecting the RPE. If this could be achieved, the combination of angiostatin and PDT might provide increased selectivity in damaging the targeted CNV with less damage to the RPE.

Intracellular events associated with photosensitizers and their subsequent activation with light are currently not well understood. PDT induces cell death by apoptosis in several cell lines,[20–24] and we wanted to characterize the mechanism of PDT-induced cell death in cell lines relevant to CNV. Lutetium Texaphyrin (Lu-Tex) is a new generation photosensitizer currently in clinical trial for the treatment of CNV, because of its favorable characteristics for clinical use, including absorption at 732 nm permitting deep tissue penetration and rapid clearance.[25] Lu-Tex/PDT appears to induce tumor involution in the murine EMT6 sarcoma model by a mixture of apoptosis and necrosis.[26] However, because PDT-induced apoptosis appears to be a function of the photosensitizer, cell line, and severity of treatment conditions these findings cannot be extended to CNV.[22,27–31]

Apoptosis involves the activation of a genetically determined programmed cell suicide that results in a morphologically distinct form of cell death characterized by cell shrinkage, nuclear condensation, DNA fragmentation, membrane reorganization, and blebbing.[32] It has been suggested that apoptosis is associated with the generation of ROS and that the product of the *bcl-2* gene protects against apoptosis by inhibiting the generation or the action of ROS.[33–36] Bcl-2 belongs to a growing family of apoptosis-regulating gene products, which may either be antagonists (Bcl-2, Bcl-x_L) or death agonists (Bax, Bak).[37] Control of cell death appears to be regulated by these interactions and by constitutive activities of the various family members.[33] It is known that several apoptotic pathways coexist in mammalian cells that are preferentially activated in a stimulus-, stage-, and context-specific and cell-type manner.[38] A proper understanding of the specific mechanism(s) involved in Lu-Tex/PDT–induced cytotoxicity in cells of relevance to CNV may permit interventions that enhance the selectivity and effectiveness of this modality.

Previously, we reported the characterization of an in vitro system for the study of Lu-Tex/PDT's effect in cell lines of relevance to CNV treatment: BRCE cells and human RPE cells (Renno et al., unpublished data, May 1999). In the present study the same system was used to investigate the possibility of an interactive cytotoxic effect of human angiostatin and Lu-Tex/PDT selective to BRCE as a means to reduce the cytotoxic effect of PDT on RPE cells. In the second part of the study, the mode of Lu-Tex/PDT–induced cell death was investigated in BRCE and RPE cell lines. In view of the special relationship among Bcl-2, PDT, and ROS, we also analyzed the constitutive expression of Bcl-2, Bcl-x_L, Bax, and Bak in BRCE and RPE cells and determined their modulation after PDT.

Materials and Methods

Cell Culture

BRCE cells (kindly provided by Patricia A. D'Amore, Schepens Eye Research Institute, Boston, MA) and human RPE cells (generous donation of Anthony P. Adamis, Massachusetts Eye and Ear Infirmary, Boston) were grown at 37°C in 5% CO_2 in Dulbecco's modified Eagle's mediums (DMEM; Sigma, St. Louis, MO), 5% heat-inactivated fetal bovine serum (FBS, Gibco, Grand Island, NY), supplemented with L-glutamine, penicillin, and streptomycin (Gibco).

Photosensitizer

Lutetium-Texaphyrin (Lu-Tex, motexafin lutetium, PCI 0123) was supplied by Alcon Research (Fort Worth, TX) as a stock solution of 2 mg/ml stable in the dark at 4°C and was used according to the manufacturer's guidelines.

Photodynamic Treatment of Cell Cultures

Cells were plated at a density of 10^5 in DMEM with 5% FBS and incubated (37°C in 5% CO_2) for 24 hours. The medium was removed and replaced by 3 μg/ml Lu-Tex in DMEM plus 5% FBS. Thirty minutes later, the cultures were exposed to timed irradiation using an argon/dye photocoagulator at 732 nm and laser delivery system (model 920; Coherent, Palo Alto, CA). Irradiance was delivered at a rate of 10 mW/cm^2 to give a total dose of 5 to 20 J/cm^2, and irradiation time ranged from 7 to 28 minutes, respectively. After irradiation, the medium was removed and replaced with complete medium. Cultures were photographed at various times after Lu-Tex/PDT using a 16× 0.32 numeric aperture on a phase-contrast inverted microscope (Diaphot; Nikon, Melville, NY).

Proliferation Assay

BRCE and RPE cells were plated at a density of 10^5 in DMEM with 5% FBS and incubated at 37°C in 5% CO_2. After 18 hours, recombinant human angiostatin (Calbiochem, La Jolla, CA) was added at a concentration of 500 ng/ml. Eighteen hours later, medium was removed and replaced by 3 μg/ml Lu-Tex in complete medium. Thirty minutes later, cells were treated with Lu-Tex/PDT at various light doses, as described. Cultures were returned to the incubator for 7 days, after which cells were dispersed in trypsin and counted in a masked fashion, and the surviving fraction was determined. Results are reported as the mean of triplicate experiments ± SD.

Preparation of Cell Lysates and Protein Determination

At various times after administration of Lu-Tex/PDT, 10^6 cells were collected by centrifugation, and the washed cell pellet was resuspended in 500 μl ice-cold lysis buffer (pH 7.5) containing 10 mM Tris, 130 mM NaCl, 1% Triton X-100, 10 mM NaF, 10 mM NaPi, 10 mM NaPPi, 16 μg/ml benzamidine, 10 μg/ml phenanthroline, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml pepstatin, and 4 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride, hydrochloride (AEBSF). Cellular lysates were stored in aliquots at −84°C for later protease activity assay or Western blot analysis. A protein assay (Coomassie Plus; Pierce, Rockford, IL) with bovine serum albumin (BSA) standard was used to assay protein concentration in cell extract.

Protease Activity

Aliquots containing 50 μg of cellular protein were incubated with 14 μm (final concentration) N-acetyl(Asp-Glu-Val-Asp)-AFC(7-amino-4-trifluoromethyl coumarin) (Ac-DEVD-AFC (PharMingen, San Diego, CA) in 1 ml protease assay buffer (pH 7.2), containing 20 mM piperazine-*N*-*N*'-bis(2-ethanesulfonic acid; PIPES), 100 mM NaCl, 10 mM dithiothreitol, 1 mM EDTA, 0.1% (wt/vol) 3-([13-cholamidopropyl] dimethylammonio)-2-hydroxy-1-propanesulfonate [CHAPS], and 10% sucrose, at 37°C for 1 hour. Fluorescence was measured using a spectrofluorometer ($\lambda_{excitation}$, 400 nm; $\lambda_{emission}$, 505 nm; model MPF-44A; Perkin-Elmer, Norwalk, CT). Cellular protein served as the blank. Results were compared with a standard curve constructed with AFC (Sigma).

Protein Electrophoresis and Western Blot Analysis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of proteins was performed with 12% SDS-polyacrylamide gels. All samples were boiled in denaturing sample buffer, and equal amounts of proteins were loaded per lane. Proteins were separated at room temperature under reducing conditions at 120 V. Western blot transfer of separated proteins was performed at room temperature, using polyvinylidene fluoride membranes at 50 mA for 1 hour. To verify equal protein loading, blots were stained with 0.1% ponceau red (Sigma) diluted in 5% acetic acid. Afterward, blots were blocked for 1 hour in Tris-buffered saline (TBS; 10 mM Tris-HCl [pH 7.5] and 150 mM NaCl) containing 5% nonfat dried milk. Next, the membranes were probed with an appropriate dilution (1:250–1:1000) of primary antibody in TBS containing 2.5% nonfat dried milk for 1 hour 30 minutes. Mouse polyclonal antibodies against Bcl-2, Bcl-x_L, Bax, and Bak were purchased from PharMingen. After incubation with primary antibody the blots were washed for 30 minutes with frequent changes of TBS, blocked in 1% nonfat dried milk in TBS for 30 minutes, and incubated in a peroxidase-coupled secondary antibody for 1 hour in TBS containing 1% nonfat dried milk. The blots were washed for 1 hour with frequent changes of TBST (TBS + 0.1% Tween). Immunoblot analysis was performed using enhanced chemiluminescence plus Western blot detection reagents (Amersham Pharmacia Biotec, Piscataway, NJ) followed by exposure to x-ray film (ML; Eastman Kodak, Rochester, NY).

Statistical Analysis

Data for all experiments were analyzed using Student's *t*-test with the level of significance set at $P \leq 0.05$.

Results

Effect of Combined Angiostatin and Lu-Tex/PDT: BRCE

To assess the effect of combining angiostatin to Lu-Tex/PDT on BRCE cell survival, cells were pretreated for 18 hours with 500 ng/ml angiostatin after which cells were treated with Lu-Tex/PDT at various fluences. Cellular survival was measured by a 1-week cellular proliferation assay. A 1-week interval was chosen rather than a shorter interval to better distinguish the lasting cytotoxic effect of the combination of angiostatin/PDT versus the short-term angiostatic effect that angiostatin exerts on the cells during the incubation period. Before testing the combination of angiostatin and Lu-Tex/PDT, we demonstrated that human angiostaton targets BRCE cells. When exposed to angiostatin alone, the proliferation assay demonstrated a 12.61% killing of BRCE cells at the angiostatin dose used. It was also observed that pre-exposing BRCE cells to angiostatin did not interfere with the subsequent cellular uptake of Lu-Tex (data not shown). More important, results showed a synergistic cytotoxic effect of angiostatin and Lu-Tex/PDT on BRCE cells at all fluences used (5, 10, and 20 J/cm^2), consistently exceeding the cytotoxicity resulting from Lu-Tex/PDT alone, angiostatin alone, or the arithmetic sum of their respective toxicities (Fig. 1a). Controls consisted of cells exposed to light only, because no dark toxicity was observed at the concentration of Lu-Tex used. Furthermore, it was observed that angiostatin was not effective in potentiating the effect of Lu-Tex/PDT if delivered after PDT (Table 1).

Effect of Combined Angiostatin and Lu-Tex/PDT: RPE

In contrast to BRCE cells, no cytotoxicity was observed when human RPE cells were treated with human angiostatin, and no interactive killing was observed after exposure to angiostatin and Lu-Tex/PDT (Fig. 1b, Table 1). When combined with angiostatin, Lu-Tex/PDT had a lethal dose (LD_{100}) of 20 J/cm^2 for BRCE cells, whereas Lu-Tex/PDT alone required 40 J/cm^2 to achieve the same effect on BRCE cells. Our previous studies have shown that at fluences of 20 and 40 J/cm^2 RPE cell survival is 43% and 21%, respectively (Renno et al., unpublished data, May 1999).

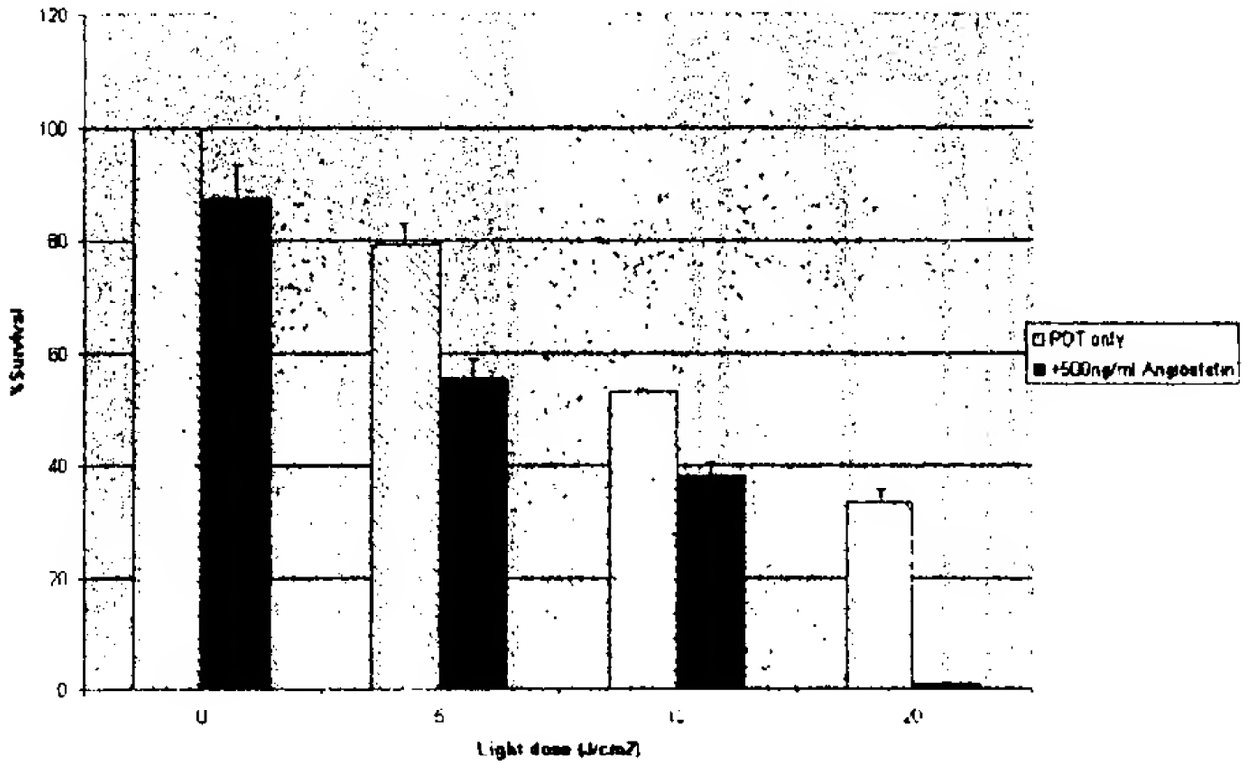
Cellular Morphology after Treatment

Although studies have shown that cells appear severely damaged immediately after PDT (Renno et al., unpublished data, May 1999), 1 week after PDT, some cells had disappeared, whereas those that remained had regained their spindle shape and their ability to attach (Figs. 2b, 2e). However, in BRCE cells that were first primed with angiostatin followed by PDT, widespread and massive cell death was observed at 1 week. Only remnants and densely refractive bodies of dying cells were seen floating in the medium (Fig. 2c). Particles were recovered and placed in fresh complete medium, but none showed any sign of reattachment or proliferation onto a new dish. It was concluded that the combination of angiostatin and Lu-Tex/PDT was lethal to BRCE cells under the conditions used. Control BRCE and RPE cells that were treated with angiostatin alone for 18 hours continued to proliferate and reached confluence (Figs. 2a, 2d). No additive effect of angiostatin to Lu-Tex/PDT was observed in RPE cells. Cells that were subjected to Lu-Tex/PDT alone or angiostatin + Lu-Tex/PDT appeared unchanged, as evidenced by the morphology (Figs. 2e, 2f).

Caspase 3-like Activation after Lu-Tex/PDT

To investigate the role of apoptosis in Lu-Tex/PDT–mediated cell death in BRCE and RPE cells, the activation of caspase 3-like (DEVD-ase) protease was monitored, as a hallmark of apoptosis. The kinetics of activation were measured spectrofluorometrically by assaying the hydrolysis of a substrate that can be cleaved only by the caspase 3-like protease family members (Ac-DEVD-AFC). Figure 3 illustrates the time course of Ac-

(a)



(b)

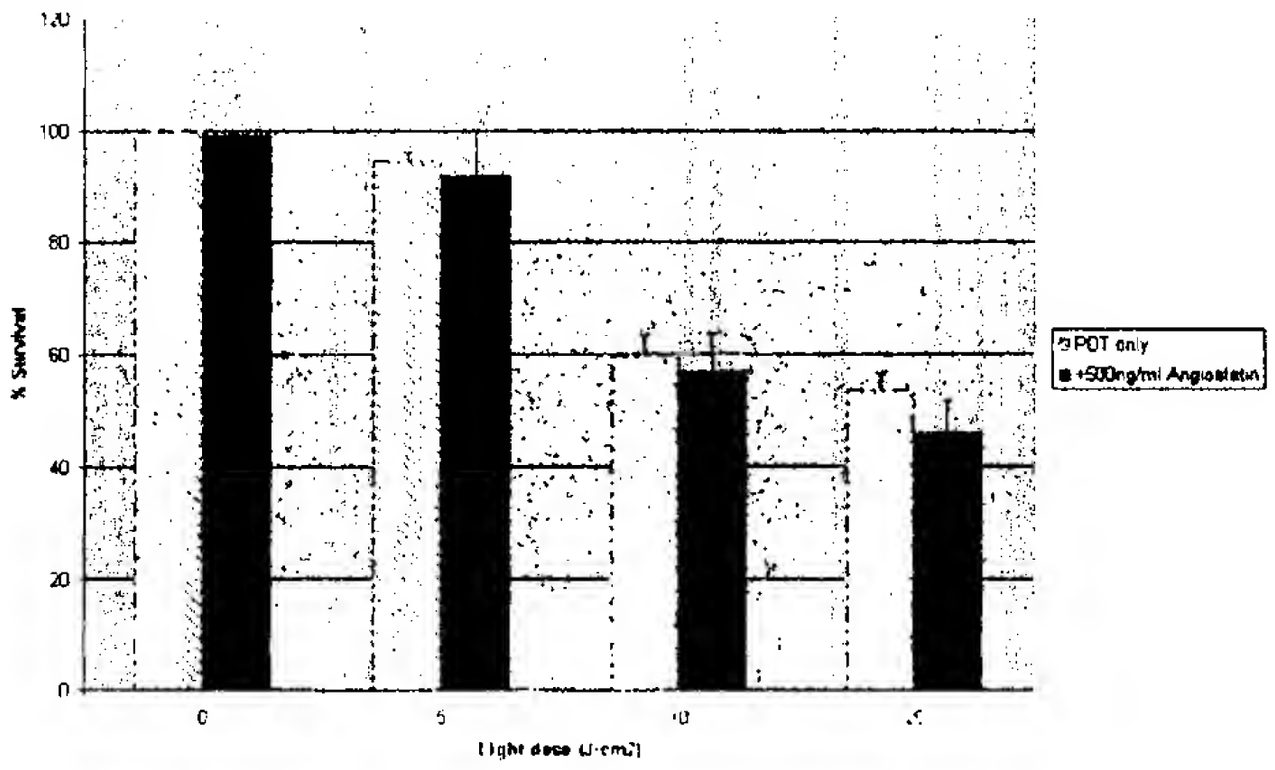


FIGURE 1. BRCE and RPE cell survival after Lu-Tex/PDT ± angiostatin. In vitro survival of (a) BRCE cells and (b) RPE cells on exposure to Lu-Tex/PDT in the presence of angiostatin. Cells were plated and exposed to angiostatin 18 hours before Lu-Tex/PDT. A 1-week proliferation assay was used to determine the surviving fraction. Data represent the mean of triplicate experiments ± SD.

TABLE 1. Summary of Cellular Survival (%) as a Function of Treatment

Cell Line	Lu-Tex/PDT*	Angiostatin	Angiostatin Followed by Lu-Tex/PDT	Lu-Tex/PDT Followed by Angiostatin
BRCE	70.13 ± 4.05 (5)	87.39 ± 5.76	55.22 ± 3.65	77.61 ± 3.52
	53.17 ± 0.52 (10)		58.11 ± 2.50	67.16 ± 3.20
	33.34 ± 2.20 (20)		0.90 ± 0.32	32.97 ± 2.20
RPE	91.55 ± 1.66 (5)	99.09 ± 0.8	91.84 ± 7.97	
	89.59 ± 3.56 (10)		86.84 ± 6.61	
	83.47 ± 3.18 (20)		85.83 ± 5.51	

The interactive in vitro antiendothelial effect of combined treatment with angiostatin and Lu-Tex/PDT are greater than additive when compared with the sum of expected effects of each treatment alone. The potentiation of Lu-Tex/PDT's effect on BRCE cells was effective with pre-exposure to angiostatin only. No effect of angiostatin was observed on RPE cells. Data are mean percentage of cellular survival ± SD.

*Fluences in parentheses are expressed in joules per square centimeter.

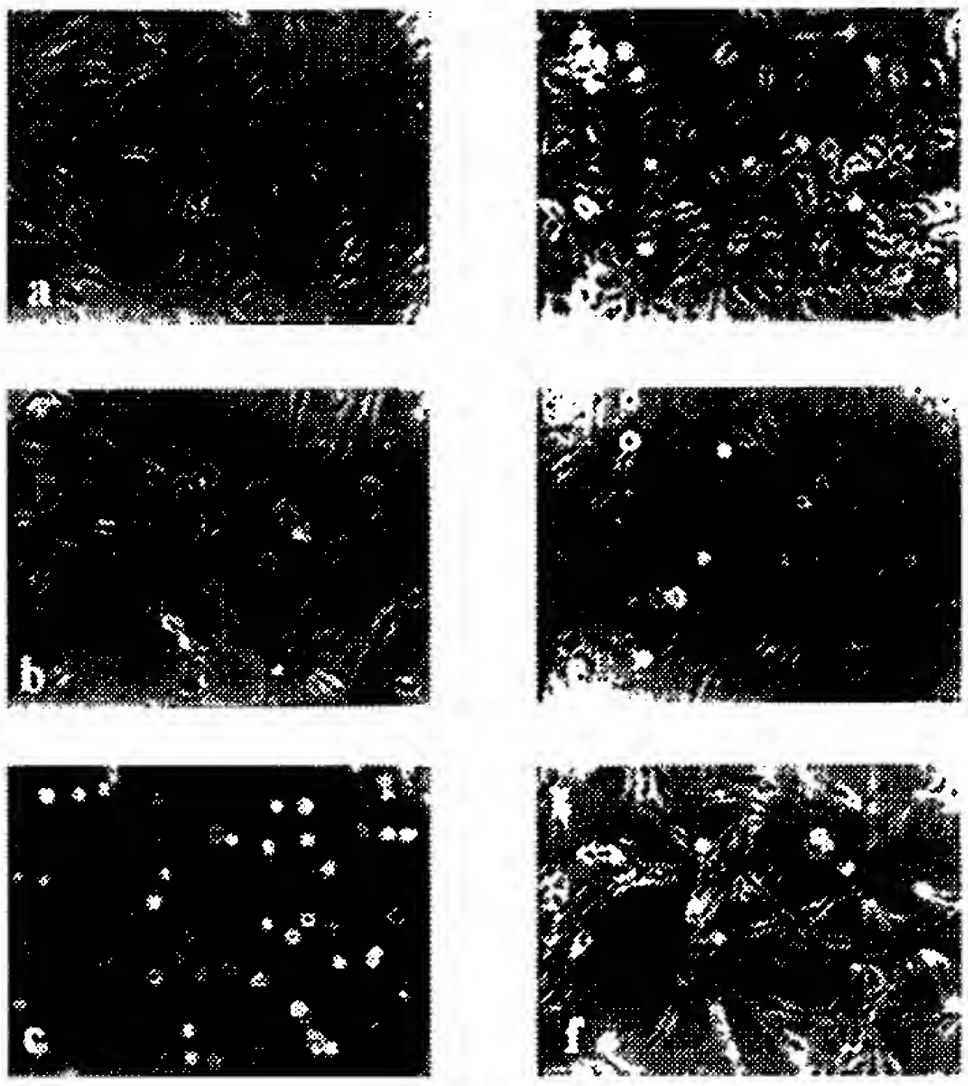


FIGURE 2. BRCE and RPE cell morphology after treatment with angiostatin + Lu-Tex/PDT (20 J/cm^2). Micrographs are of representative fields from a 1-week proliferation assay of BRCE and RPE cells after treatment. BRCE cells treated with angiostatin only (**a**), Lu-Tex/PDT only (**b**), and angiostatin + Lu-Tex/PDT (**c**). RPE cells treated with angiostatin only (**d**), Lu-Tex/PDT only (**e**), and angiostatin + Lu-Tex/PDT (**f**). Magnification, ×16.

DEVD-AFC cleavage after Lu-Tex/PDT at three different light doses in BRCE and RPE cells. Results show a rapid elevation of caspase 3-like activity immediately after Lu-Tex/PDT—as early as 10 minutes after Lu-Tex/PDT and peaking at 30 minutes—in both BRCE and RPE cells and at all doses used. Clearly, the rate of entry into apoptosis was time and dose dependent in each cell line. However, the amount of caspase 3-like activation was always significantly higher in BRCE cells than in RPE cells. Furthermore, whereas at 10 and 20 J/cm^2 the amount of caspase3-like activation was increased by approximately 50% in BRCE cells compared with RPE cells, at 40 J/cm^2 (equivalent to the LD_{100} for BRCE cells), the levels in BRCE cells were five times those in RPE cells.

Caspase 3-like Activation after Angiostatin + Lu-Tex/PDT

To examine the effect of combining angiostatin and Lu-Tex/PDT on DEVD-ase activation in BRCE cells, cells were treated with angiostatin alone, Lu-Tex/PDT alone, and angiostatin + Lu-Tex/PDT, after which caspase 3-like activity was assayed as described. Fluences of 20 and 40 J/cm^2 were used, corresponding to an LD_{100} of combination angiostatin + Lu-Tex/PDT and Lu-Tex/PDT alone, respectively. Results demonstrated that the combination of angiostatin + Lu-Tex/PDT induced a statistically significant increase of caspase 3-like activity compared with Lu-Tex/PDT alone, when using a fluence of 20 J/cm^2 (Fig. 4). However, although both Lu-Tex/PDT (40 J/cm^2) and the combination of angiostatin + Lu-Tex/PDT (20 J/cm^2) resulted in 100% lethality to BRCE cells, Lu-Tex/PDT (40 J/cm^2) resulted in increased levels of caspase 3-like activity compared with angiostatin + Lu-Tex/PDT (20 J/cm2). As in the case of BRCE cells treated with Lu-Tex/PDT alone, the rate of entry into apoptosis of BRCE cells treated with combination of angiostatin + Lu-Tex/PDT was time dependent. Nevertheless, the time courses differed significantly, in that the induction of caspase 3-like activation occurred abruptly and more rapidly as a result of angiostatin + Lu-Tex/PDT, peaking at 30 minutes and reaching minimum levels at 90 minutes after treatment.

Modulation of Bcl-2 Family Members after Lu-Tex/PDT

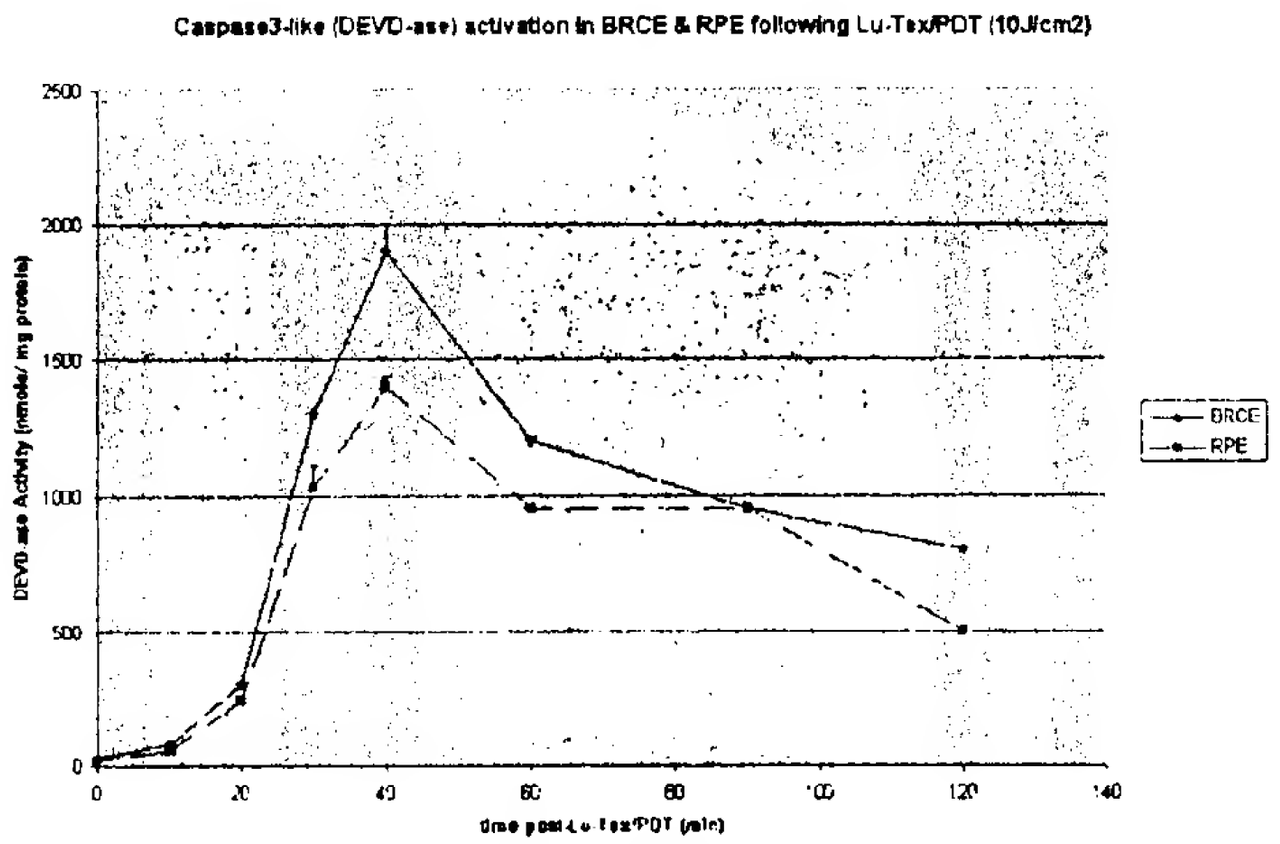
To evaluate the expression of Bcl-2 family members in BRCE and RPE cells after Lu-Tex/PDT, BRCE and RPE cells were subjected to Lu-Tex/PDT, and resultant cellular lysates were subjected to Western blot analysis for detection of the anti-apoptotic Bcl-2, Bcl-x_L, and proapoptotic Bax and Bak. Results showed a differential expression of members of Bcl-2 family members in BRCE and RPE cells: Bcl-2 and Bax were detected in BRCE cells, whereas Bcl-x_L and Bak were detected in RPE cells (Table 2). After Lu-Tex/PDT at LD_{50}, downregulation of Bcl-2 and upregulation of Bax was observed in BRCE cells, resulting in an increase of the cellular ratio of Bax to Bcl-2 protein (Fig. 5a). In RPE cells, there was an upregulation of both Bcl-x_L and Bak up to 4 hours after PDT, after which Bcl-x_L levels reached a plateau, and Bak level started to decline (Fig. 5b). Furthermore, our results demonstrated that the upregulation of Bax in BRCE cells was dose dependent; however, the upregulation of its proapoptotic counterpart Bak in RPE cells exhibited dose dependence only until 20 J/cm^2, after which it began to decline (Fig. 5c).

DISCUSSION

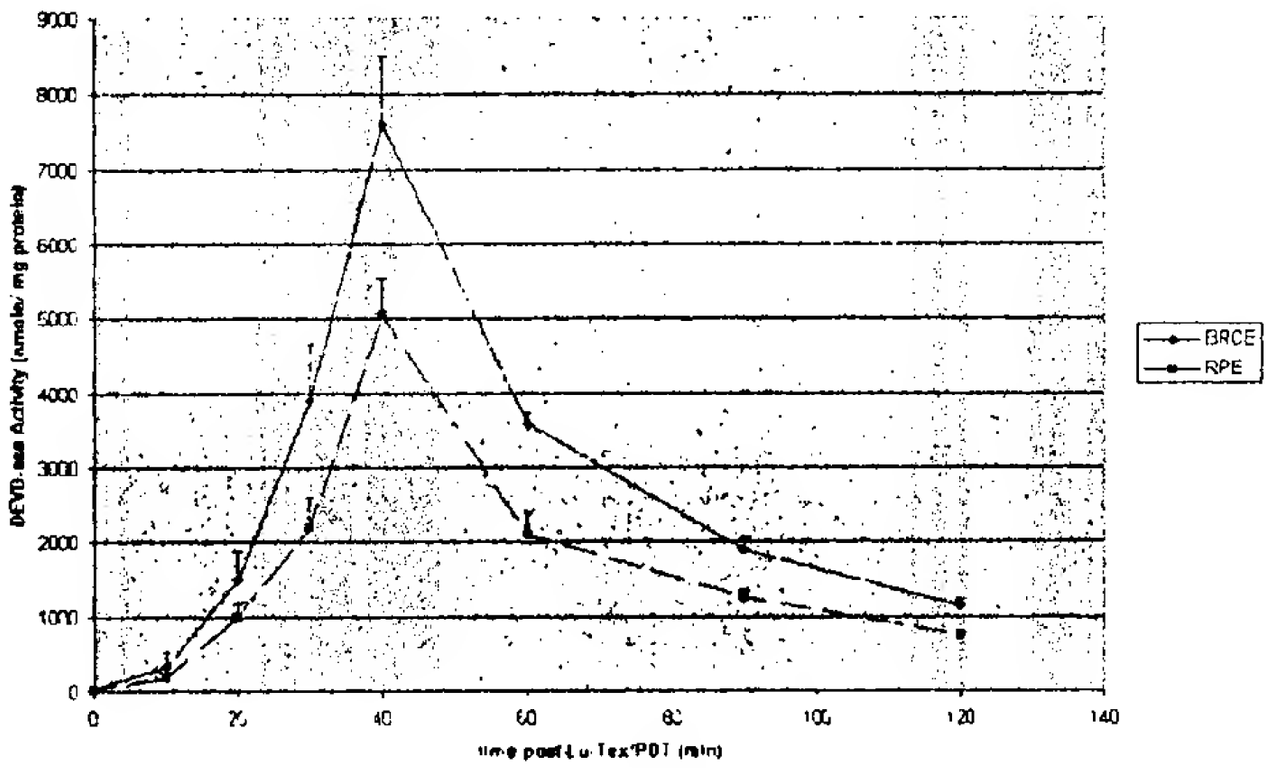
The promising results witnessed with PDT for the treatment of CNV along with some observed side effects sustained by the RPE in the course of treatment, prompted us to seek different strategies to improve the efficacy and selectivity of PDT to CNV. One such strategy was to investigate a role for angiostatin as a potential adjuvant of Lu-Tex/PDT because of its established property as a specific inducer of quiescence in certain endothelial cell lines. Another approach was to investigate the mode of Lu-Tex/PDT–induced cytotoxicity in BRCE and RPE cells as a preliminary step for the design of treatments that might help modulate specifically these effects at the cellular level.

Our data showed a specific antiproliferative effect of angiostatin on retinal capillary endothelial cells as demonstrated by the reduction in cell number in a 1-week proliferation assay. In contrast, no effect of angiostatin was observed on RPE. Thus, our work adds BRCE cells to the list of endothelial cell lines already known to be specifically targeted by angiostatin: bovine adrenal cortex microvascular, bovine adrenal cortex capillary, bovine aortic, human umbilical vein, and human dermal microvascular endothelium.[38,39] In our study, BRCE cells were used as a representative capillary endothelial line of the posterior segment to test the antiangiogenic effect of angiostatin, because angiostatin does not seem to rely on specific cell surface antigen recognition to exert its action on the endothelium. Therefore, it seems reasonable to assume that angiostatin would have similar effects on the choriocapillaris

(a)



(b)



(c)

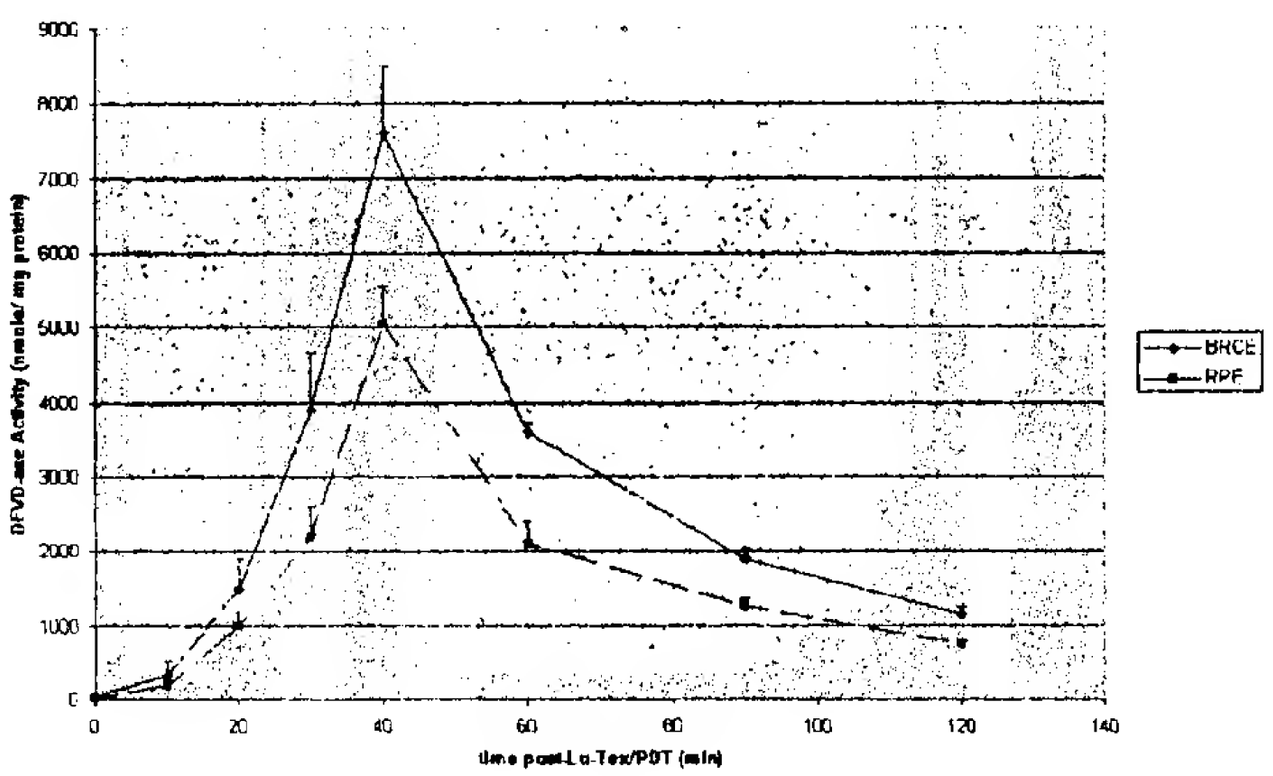


FIGURE 3. Kinetics of caspase 3-like activation after Lu-Tex/PDT in BRCE and RPE cells. BRCE and RPE cells were exposed to Lu-Tex/PDT at fluences of (a) 10, (b) 20, and (c) 40 J/cm^2. At the indicated times thereafter, cells were collected and lysed. Aliquots (50 μg of protein) were incubated with Ac-DEVD-AFC at 37°C for 30 minutes. The amount of fluorochrome released was determined by comparison with an AFC standard curve in lysis buffer. Data represent the means from three independent experiments.

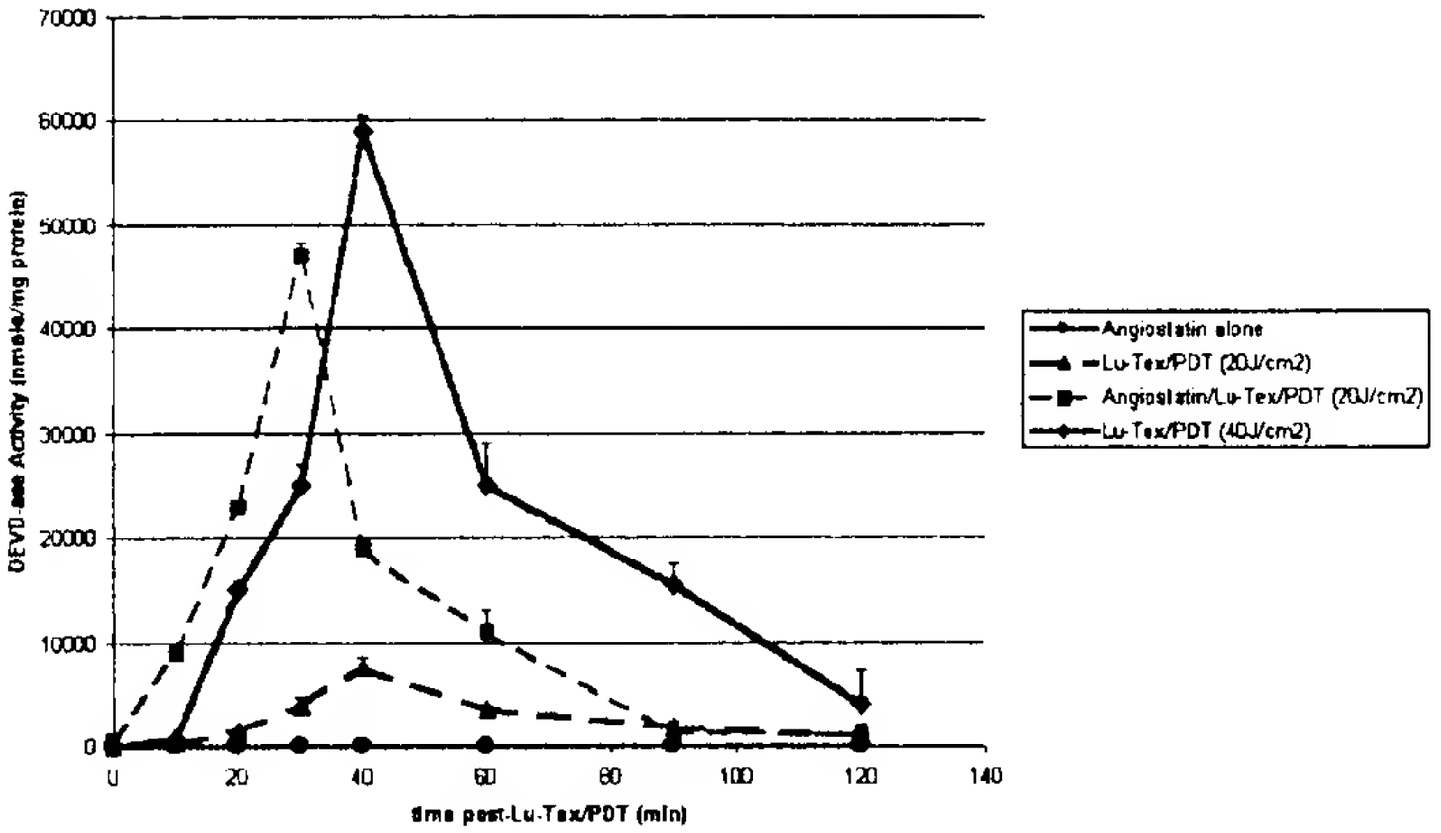


FIGURE 4. Caspase 3-like activity in BRCE cells after angiostatin + Lu-Tex/PDT versus Lu-Tex/PDT alone. BRCE cells were exposed to angiostatin (500 ng/ml) alone, Lu-Tex/PDT (20 J/cm^2, 40 J/cm^2) alone, and angiostatin + Lu-Tex/PDT. At the indicated times thereafter, cells were collected and lysed. Aliquots (50 μg of protein) were incubated with Ac-DEVD-AFC at 37°C for 30 minutes. The amount of fluorochrome released was determined by comparison with an AFC standard curve in lysis buffer. Data represent the means from three independent experiments

and retinal and choroidal neovascular endothelium. Moreover, in culture many of the differences between the choriocapillaris and retinal capillary endothelium are lost. Because angiostatin has a cytostatic rather than cytocidal effect, it could be expected it to have a selective effect on proliferating versus resting endothelium. In addition, tissue culture is thought to more closely represent proliferating tissue such as CNV than resting tissue. The finding that angiostatin induced apoptosis in BRCE cells suggests that cell death may contribute to the overall reduction of cell number; however, little is known concerning the exact antiangiogenic mechanism of angiostatin.[39]

Our in vitro studies showed that Lu-Tex/PDT and angiostatin had combined cytotoxic effects on retinal capillary endothelial cells but not pigment epithelial cells. However, when angiostatin were administered after PDT, the combination did not potentiate the effects of PDT. The efficacy of a photosensitizer is intimately related to its subcellular distribution.[40–42] Although angiostatin did not affect the intracellular incorporation of Lu-Tex, this does not exclude the possibility that it may induce a redistribution of the dye to subcellular compartments whereby its potency of action is enhanced. In the combination of angiostatin before Lu-Tex/PDT, a fluence of 20 J/cm^2 sufficed to achieve nearly 100% mortality of BRCE cells. In the absence of angiostatin, a light dose of 40 J/cm^2 would be required to achieve this level of cytotoxicity. At the light dose of 20 J/cm^2, RPE cells survival after PDT was improved by 20%. The results of our experiments thus support the potential of combining angiostatin with Lu-Tex/PDT to improve CNV eradication and decrease deleterious effects on the RPE cells. Work is currently under way in our laboratory to test the combination of angiostatin and PDT in small animal models of laser-induced CNV.

In our study, Lu-Tex/PDT induced caspase 3-like activation in both BRCE and RPE cells in a dose- and time-dependent

TABLE 2. Summary of Immunodetection of Bcl_2 Family Members in BRCE and RPE Cells

	Cell Line	
Bcl_2 Family Member	**BRCE**	**RPE**
Bcl_2	+	–
$Bcl\text{-}x_L$		+
Bax	+	
Bak		+

Detectable (+) or undetectable (–)

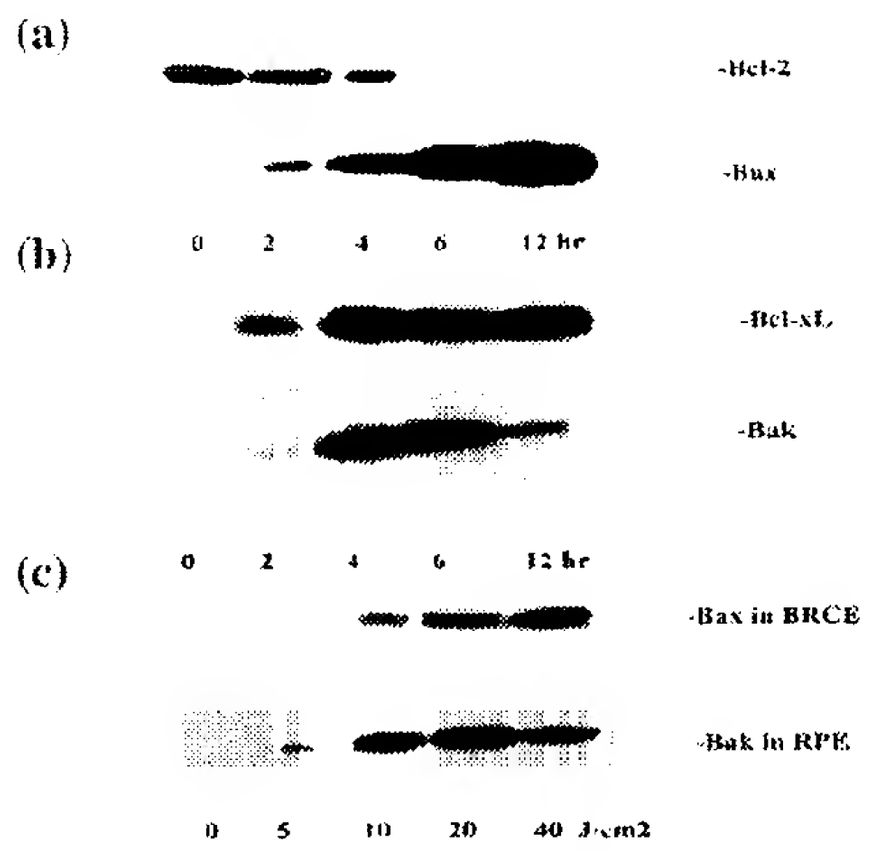


FIGURE 5. Expression of Bcl-2, Bcl-x_L, Bax, and Bak in BRCE and RPE cells after Lu-Tex/PDT. (**a**) BRCE and (**b**) RPE cells were treated with the 50% lethal dose (LD_{50}) of Lu-Tex/PDT. At the indicated time points after PDT, whole cell extracts were obtained and analyzed by SDS-PAGE followed by Western blot analysis using antibodies to Bcl-2, Bcl-xL, Bax, and Bak. In BRCE cells, upregulation of Bax and downregulation of Bcl-2 were observed over 12 hours. In RPE cells, upregulation of Bcl-x_L was observed along with peak upregulation of Bak up to 4 hours followed by its progressive decline. (**c**) After incremental doses of PDT, BRCE and RPE cellular lysates were obtained at 4 hours after treatment and analyzed by SDS-PAGE followed by Western blot analysis using antibodies to Bax and Bak. In BRCE cells, Bax was upregulated in a dose-dependent fashion. In RPE cells, the level of Bak plateaued at a fluence of 20 J/cm^2.

fashion, suggesting that apoptosis is a mediator of Lu-Tex/PDT cytotoxicity in these cell lines. Furthermore, our data indicate that Lu-Tex/PDT induced apoptosis in BRCE cells through the modulation of Bcl-2 and Bax in a dose- and time-dependent fashion and in RPE cells through the modulation of Bcl-x_L and Bak. However, Lu-Tex/PDT may cause alternative death modes as was shown when tested in vivo in the murine EMT6 sarcoma model,[26] and based on the evidence that photofrin/PDT induces apoptosis or necrosis in a monkey kidney cell line (CV1) depending on the incubation protocol.[31] Therefore, in vivo confirmation of such a finding is required in CNV models

The time course of caspase 3 activation after PDT, as noted by other investigators, varies according to cell lines and photosensitizers,[45] ranging from minutes to hours: less than 10 minutes for LY-R,[20] 20 minutes for BRCE and RPE cells, and hours for Hela cells.[44] However, unlike other reports, the kinetics in our study in BRCE and RPE cells were constant when the PDT light dose was varied. Furthermore, whereas the magnitude of DEVD-ase activity was 50% higher in BRCE versus RPE cells at fluences of 10 and 20 J/cm^2 it nearly exceeded 500% at LD_{100} (40 J/cm^2); this however does not necessarily correlate with the number of apoptotic cells involved. The possible explanations include the fact that individual intracellular levels of caspase 3-like are unknown, as is the threshold of DEVD-ase activation required for cellular death. Yet, at all times after PDT, there was an upregulation of the antiapoptotic Bcl x_L levels in RPE cells. Concomitantly, at 4 hours after treatment, the levels of the proapoptotic Bak started declining after its initial upregulation. Furthermore, after incremental PDT doses, the proapoptotic Bak was upregulated in RPE cells until 20 J/cm^2 after which Bak levels started declining despite an increase of PDT dose to 40 J/cm^2. It is thus conceivable to think of a protective survival response being mounted in RPE cells at these lethal doses to counteract the apoptotic trigger. Such a hypothesis is further supported by the histologic evidence of RPE cell recovery after PDT in vivo[15,45] and by reports from other investigators that overexpression of antiapoptotic Bcl-2 family members renders cells partially resistant to PDT[36] and inhibits the activation of caspase-3 after PDT.[47] Reversibly, antisense Bcl-2 retrovirus increases the cells' sensitivity to PDT.[48]

The present data show that the combination of angiostatin and Lu-Tex/PDT in BRCE cells resulted in an increase in DEVD-ase activity compared with the same dose of Lu-Tex/PDT applied alone. This suggests that the potentiating action of angiostatin on the effect of Lu-Tex/PDT in BRCE cells proceeds through apoptosis. Even if angiostatin induces a subcellular localization of Lu-Tex, such redistribution remains confined to cellular compartments (mitochondria, lysosomes, and melanosomes) where their mode of action ensues through apoptosis. However, the time course of caspase 3-like activity for angiostatin + Lu-Tex/PDT differed from that of Lu-Tex/PDT alone, in that it proceeded faster without latency and peaked as soon as 20 minutes after Lu-Tex/PDT. An explanation for the latter could that the apoptotic cascade was already primed by preincubation with angiostatin first, and thus the application of Lu-Tex/PDT benefited from an already lowered threshold of activation to rapidly amplify the apoptotic response. However, this does not exclude the possibility of the interplay of more than one apoptotic pathway, especially because PDT is known to initiate cytotoxicity through the generation of ROS,[11] whereas angiostatin was recently shown to act on human endothelial cells by binding to the a-subunit of adenosine triphosphate (ATP) synthase present on the cell surface.[49] Furthermore, whereas angiostatin + Lu-Tex/PDT (20 J/cm^2) resulted in a 100% lethality of BRCE cells as did Lu-Tex/PDT (40 J/cm^2) alone, the levels of DEVD-ase activation were significantly higher in the former regimen. This supports the hypothesis that Lu-Tex/PDT and angiostatin + Lu-Tex/PDT operate through different apoptotic pathways in BRCE cells.

In summary, in our study angiostatin exhibited an antiproliferative effect on BRCE cells and had no notable effect on RPE cells. Angiostatin combined with Lu-Tex/PDT potentiated cytotoxicity in BRCE cells. Lu-Tex/PDT induced rapid caspase-dependent apoptosis in BRCE and RPE cells. Furthermore, Lu-Tex/PDT induced apoptosis through the selective and differential modulation of members of the Bcl-2 family in BRCE and RPE cells

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FIBRONECTIN FRAGMENTS IN ANGIOGE[illegible]S ((Maria B. Grant[1], Sergio Caballero[1], Roy W. Tarnuzzer[1], Kathryn E. Bass[2], David M. Bush[1], and Polyxenie E. Spoerri[1])) [1]University of Florida, Gainesville, FL, and [2]University of California, San Francisco, CA.

Purpose. We investigated the expression of two matrix metalloproteases (MMP-2 and MMP-9) and their inhibitors, TIMP-1 and TIMP-2 following varying glucose exposures in human retinal endothelial cells (HREC). Fibronectin (Fn) is a key ECM component regulated by glucose. In these cultures, we examined the generation of fibronectin fragments (Fn-f) and tested the effect of selected Fn-f on proliferation and migration. **Methods.** HREC from nondiabetic (ND) and diabetic (D) donors were exposed to 5 mM or 30 mM glucose for 8 or 24 h. Secreted MMP activity was measured by gelatin zymography. Competition based quantitative RT-PCR was used to detect mRNAs coding Fn, MMP-2, MMP-9, and their inhibitors. Western blotting was used to identify specific proteases and associated peptides in conditioned medium (CM). The effects of Fn-f on proliferation was determined by BrdU incorporation. Their effect on migration was assessed using modified Boyden chambers. **Results.** CM from all cultures expressed a proteolytic band migrating at 72 kDa, unchanged by glucose exposure, and confirmed by Western blot analysis as the proenzyme form of MMP-2. In contrast no MMP-9 expression was detected. CM from ND HREC cultures demonstrated a proteolytic activity migrating at 90 kDa in cells exposed to 30 mM glucose, but not to 5 mM glucose. This same 90 kDa activity was seen in CM from HREC cultures of D origin in the presence of both low and high glucose conditions. The proteolytic activity at 90 kDa represented a Fn-f bound to MMP-2. The binding of this fragment to the proform of MMP-2 prevented APMA activation. In D and ND HREC, MMP-2, TIMP-1 and TIMP-2 mRNAs were expressed constitutively and were unchanged by exposure to 30 mM glucose. Similarly, fibronectin mRNA expression was not changed by glucose in either D or ND HREC. A 30 kDa tryptic Fn-f stimulated endothelial cell migration in a dose-dependent manner ($p<0.01$). In contrast, a 45 kDa chymotryptic fragment from the gelatin/collagen domain of Fn inhibited HREC proliferation 20-fold, but stimulated HREC migration 4.5 fold over basal ($p<0.01$). Fn-f of 120 kDa size, which contained the heparin and cell binding domains, was a potent stimulator of HREC proliferation, inducing a 70 fold increase at 24 h and 10 fold increase in migration $p<0.001$). **Conclusion.** Regulation by glucose of ECM components such as fibronectin may influence angiogenesis by the generation of fragments which can modulate proliferation, migration, and protease activation. Production of Fn-f may be specifically relevant to the angiogenesis observed in proliferative diabetic retinopathy NIH EY07739 NONE

4472 — 6:15

PLACENTAL GROWTH FACTOR LOCALISATION IN DIABETIC RETINAS AND PRERETINAL MEMBRANES
((M. Boulton[1], D. Foreman[1], D. McLeod[1], H. Weich[1], A. Khaliq[2] and A. Ahmed[2])) [1]Manchester Eye Hospital, Manchester, UK; [2]Reproductive Pathophysiology Group, Birmingham Womans Hospital, Birmingham, UK; [3]Institute of Molecular Biology, University of Frieburg, Germany

Purpose. To determine the distribution of a recently identified member of the VEGF family, namely placental growth factor (PlGF), at different stages of diabetic retinopathy. **Method.** Immunohistochemical localisation of PlGF was carried out using a rabbit anti-serum raised against a 20 amino acid N terminal sequence to PLGF (no cross-reactivity occurred with any VEGF isoform as determined by Western blotting) on specimens of normal human retina, diabetic retinas (either with no overt retinopathy or with active proliferative retinopathy), and preretinal membranes. The distribution and intensity of staining for PlGF reactive protein was recorded and compared with immunostaining for $VEGF_{165}$. **Results.** Immunostaining for PlGF was absent from the normal retina but was present in the majority of diabetic retinas with no overt retinopathy, especially in the thickened basement membranes of the retinal vessels. In all retinas with active neovascularisation, immunostaining for PlGF was intense, especially associated with intraretinal vessels adjacent to areas of active preretinal neovascularisation. PlGF staining was intense in all excised PDR membranes being localised to both the vessels and the surrounding matrix. The staining pattern was similar to that observed for $VEGF_{165}$. Avascular PVR membranes did not stain for PlGF although serial sections stained for VEGF. **Conclusions.** This is the first study to report the localisation of PlGF in the eye and suggests that PlGF may be an important factor in retinal angiogenesis.
Supported by the British Diabetic Association. None.

4473 — 6:30

$\alpha_v\beta_3$, $\alpha_v\beta_5$, AND OSTEOPONTIN IMMUNOSTAINING IN EXPERIMENTAL CHOROIDAL NEOVASCULARIZATION IN THE MONKEY . ((M Corjay[1], D Husain[2], J. Stoltenborg[1], S. Diamond[1], N Michaud[3], JW Miller[2])) DuPont Merck Research Laboratories, Wilmington, Delaware; Massachusetts Eye and Ear Infirmary[2], Mass. General Hospital[1], Harvard Medical School, Boston, MA.

Purpose. $\alpha_v\beta_3$ and $\alpha_v\beta_5$ are integrin receptors which have been demonstrated in ocular neovascularization *in vivo*. Osteopontin is a ligand for these receptors. The aim of this study was to investigate the timing and distribution of expression of these molecules in a monkey model of choroidal neovascularization (CNV). **Methods.** CNV developed in 4 cynomolgus monkey eyes following argon laser injury. CNV was followed by fundus photography and fluorescein angiography. Immunostaining was performed on paraffin sections using an anti-$\alpha_v\beta_3$ monoclonal (LM609) antibody, an anti-$\alpha_v\beta_5$ monoclonal (P1F6) antibody, and a guinea pig polyclonal antibody to osteopontin on specimens obtained 1, 7, 14, and 21 days post laser, and compared to staining of a control eye. **Results.** Mild staining for $\alpha_v\beta_3$ and $\alpha_v\beta_5$ was seen in the control eye in the ganglion cell layer (GCL), the inner (IPL) and outer plexiform layerers (OPL), the retinal pigment epithelium (RPE), and the vessel walls of choroidal vessels. Increased staining of the RPE near the laser site was noted for $\alpha_v\beta_3$, but not for $\alpha_v\beta_5$ on days 1 and 7. Neovascularization arising from the laser site by day 14 showed moderate staining for $\alpha_v\beta_3$ and osteopontin at the edges of the CNV, in the RPE and in the capillaries of the CNV. On day 21, bright staining for $\alpha_v\beta_3$, $\alpha_v\beta_5$, and osteopontin were noted within the CNV and RPE. **Conclusions.** $\alpha_v\beta_3$ and $\alpha_v\beta_5$ can be demonstrated in non-vascular cells in normal and neovascular tissue. Expression of $\alpha_v\beta_3$, $\alpha_v\beta_5$, and osteopontin are temporally and spatially regulated during the development of experimental CNV.

Supported in part by Research to Prevent Blindness. E

4474 — 6:45

ADENOSINE IN RETINAL VASCULOGENESIS AND OXYGEN-INDUCED RETINOPATHY ((G.A. Lutty, C. Merges, M. Kunz, and D.S. McLeod)) Wilmer Ophthalmological Institute, Johns Hopkins Hospital, Baltimore, MD.

Purpose: We examined the distribution and relative levels of adenosine (ADO) and 5' nucleotidase in neonatal dog inner retina during normal vasculogenesis and oxygen-induced retinopathy (OIR). 5' nucleotidase (5'N) is a major source of adenosine in most tissues. Adenosine is a potent vasodilator that is angiogenic in other systems; recent data suggests that it may control VEGF expression. **Methods:** Twenty seven animals ranging in age from 1 to 22 days of age were used in this study. Adenosine immunolocalization was performed on frozen sections with an antibody against adenosine conjugated to levulinic acid using a streptavidin peroxidase technique. Triplicate air control animals at different postnatal ages and triplicate oxygen exposed animals at different time points during or after oxygen insult were examined. Adenosine immunoreaction product (ADORP) was analyzed in triplicate sections from each animal using microdensitometry. Adjacent sections were incubated for von Willebrand factor immunoreactivity and 5'N enzyme activity. **Results:** During normal vasculogenesis, ADORP was most prominent within the inner retina. The peak of immunoreactivity was located at the border of vascularized retina throughout the period of primary retinal vasculogenesis (1-15 days of age). At 22 days when vasculogenesis was complete, ADORP levels decreased within the inner retina. 5'N activity was localized to Muller cell processes in inner retina and decreased after vasculogenesis was complete. In animals sacrificed after 4 days of oxygen breathing, the vaso-obliterative stage of OIR, ADORP and 5'N activity was reduced throughout the retina. During the vasoproliferative stage ADORP was markedly elevated at the edge of reforming vasculature as well as throughout the more posterior inner retina where 5'N activity was elevated ADORP was also elevated in preretinal neovascularization. **Conclusions:** Peak adenosine levels in the inner retina correlate temporally with active vasculogenesis. Adenosine and 5'N levels are reduced in hyperoxia and then rebound above normal levels during the vasoproliferative stage of oxygen-induced retinopathy.
Supported by NIH grants EY09357 (GL) and EY01765 (Wilmer Institute). G. Lutty is an American Heart Association Established Investigator. Proprietary interests: none.

4475 — 7:00

VASCULAR DEVELOPMENT IN HUMAN RETINA: MECHANISMS & TOPOGRAPHY
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Purpose: To characterise the cellular processes and topography of vascularisation in human retinae. **Methods**: Human foetal eyes, ranging in age from 14-38 embryonic weeks (W) were collected in China in accordance with the guidelines set forth in the Declaration of Helsinki. The various stages of vascularisation were visualised using Nissl stained wholemounts and anti-CD34 immunohistochemistry. **Results**: The first process in the vascularisation of the retina, prior to 15W, was the migration of large numbers of spindle-shaped mesenchymal precursor cells from the optic disc. These precursor cells proliferate and differentiate to produce solid chords of endothelial cells (EC) which become patent to form an immature vascular tree centred over the optic disc beginning from W15. Growth of the inner plexus was associated with the extension of filopodia followed by dilatation of appropriate filopodia to form a vascular segment. With maturation there is selection of major channels and significant retraction of excess vascular segments. Retraction is via the withdrawal EC from neighbouring cells followed by programmed cell death. The formation of the outer vascular plexus occurs via the extension of capillary sized buds from the existing inner vessels. The first outer vessels were apparent around the incipient fovea between W 25-26. Fine radial peripapillary capillaries (RPC's) were evident in the nerve fibre layer from W 21. **Conclusions**: We conclude that formation of the inner retinal plexus in human takes place via the 3 stage process of vasculogenesis, involving mesenchymal precursor cell invasion, EC differentiation and proliferation to form a patent vascular plexus, followed by retraction of excess capillary segments and maturation of the vascular tree. In contrast, the perifoveal vessels, the outer plexus and the RPC's are formed via the budding of capillary sized vessels, via the process of angiogenesis. The timing and topography of vessel growth is coincident with the onset of photoreceptor activity and consequential increase in metabolic demand, and is consistent with our previous hypothesis, that 'physiological hypoxia' stimulates vasoproliferation in the human retina. These normative data could assist in the administration of supplemental oxygen therapy to premature infants.
NH&MRC (Australia), R.G. Arnott Foundation, Baxter Perpetual Trust None

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Liposomal Benzoporphyrin Derivative Verteporfin Photodynamic Therapy

Selective Treatment of Choroidal Neovascularization in Monkeys

Michal Kramer, MD,[1] Joan W. Miller, MD,[1] Norman Michaud, MS,[2] Rachel S Moulton, BS,[1] Tayyaba Hasan, PhD,[2] Thomas J. Flotte, MD,[2] Evangelos S. Gragoudas, MD[1]

Purpose: The authors have previously shown that photodynamic therapy (PDT) using lipoprotein-delivered benzoporphyrin derivative mono-acid (BPD) effectively closed experimental choroidal neovascularization (CNV). In the current study, the authors used a clinical preparation, liposomal BPD verteporfin in the same model, with experiments designed to establish optimal dye and light doses, and the timing of laser light irradiation after dye injection, for effective and selective closure of CNV.

Methods: Experimental CNV was induced in the maculae of cynomolgus monkeys. Liposomal BPD verteporfin was injected intravenously at doses of 1.0, 0.5, 0.375, and 0.25 mg/kg. Laser light at 692 nm then was applied to CNV, with an irradiance of 600 mW/cm^2 and fluence of 150 J/cm^2, at various times after dye injection, ranging from 5 to 120 minutes. Treatment effect was assessed by fundus photography and fluorescein angiography and confirmed by light and electron microscopy. The PDT of experimental CNV was studied to assess efficacy; PDT performance on normal eyes was studied to investigate selectivity.

Results: The CNV closure was demonstrated by fluorescein angiography and histopathologic findings at all tested dye doses. A dye dose of 0.375 mg/kg, with laser light irradiation applied 20 to 50 minutes after dye injection, optimized CNV closure with minimal retinal and choroidal damage. No major local adverse effects were noted, and the drug was well tolerated systematically.

Conclusions: Liposomal BPD verteporfin is a potent photosensitizer, and PDT using this dye is a potentially effective and selective treatment for CNV.

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Neovascularization in different locations within the eye is a clinical manifestation of many ophthalmic diseases, including degenerative, inflammatory, and ischemic con-

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Supported in part by Quadra Logic Technologies, Inc (Vancouver, British Columbia, Canada.

The Massachusetts Eye and Ear Infirmary has a proprietary interest in this technology under a research agreement with Coherent, Inc, and as part of a patent application. Drs. Miller and Gragoudas are participants in this agreement and application under the established guidelines of Harvard Medical School.

Reprint requests to Joan W. Miller, MD, Laser Research Laboratory, Massachusetts Eye and Ear Infirmary, 243 Charles St, Boston, MA 02114.

ditions. Choroidal neovascularization (CNV) leads to severe visual loss in patients with age-related macular degeneration, the leading cause of legal blindness in patients older than 65 years.[1–3] The currently available treatment consists of thermal laser photocoagulation, which results in full thickness retinal damage.[4] This treatment is still unsatisfactory, because of the resulting visual loss when treatment involves the fovea and the high recurrence rate.[5–7]

Photodynamic therapy (PDT) may offer selective eradication of the neovascular membrane while producing minimal damage to retinal and choroidal tissues. This treatment modality uses low-intensity light at a wavelength within the absorption band of the injected dye to irradiate photosensitized tissues and cause local cytotoxic effects by photochemical reactions. The irradiated photosensitizer is transformed to its triplet state and produces singlet oxygen particles that cause damage to several cellular targets, including cell and mitochondrial membranes, lysosomes, and nuclear components.[8,9] Previous investigations have demonstrated selective accumulation of certain photosensitizers in tumors. In addition, there is evidence that vascular damage plays a major role in tumor destruction induced by PDT.[10–13] These data suggest that neovascular tissue might be targeted in other angiogenic conditions, such as ocular neovascularization, arthritic pannus, and psoriasis.

The photosensitizer under investigation, benzoporphyrin derivative mono-acid (BPD), is a synthetic chlorin-like porphyrin, which has a light absorption peak at 692 nm. In previous investigations, BPD was complexed with low-density lipoprotein (LDL) to enhance its delivery to neovascular and tumor tissue and its PDT effect.[14,15] Neovasculature may selectively accumulate lipoprotein-associated photosensitizers because of increased LDL receptors in rapidly proliferating endothelium and increased LDL transport across the endothelium of permeable vessels.[16–18] Using lipoprotein-delivered BPD, we have shown previously that PDT effectively closes experimental CNV in monkeys.[14]

In this study, we used an improved preparation of BPD, which uses liposomes as its delivery system. The liposome is a unilamellar phospholipid vesicle based on dimyristoyl phosphatidyl choline and egg phosphatidyl glycerol. The lipophilicity of BPD resulted in 100% efficiency of incorporation into the liposome. This dye formulation partitions more readily into the plasma lipoproteins, reaches higher levels in tumor tissue, and has been shown to be a more potent photosensitizer in vivo.[19] In addition, it is readily reconstituted to a stable liquid form and results in an accurate, reliable concentration. This preparation is currently in clinical trial for the treatment of malignant skin tumors.[20]

We studied the dye dosimetry and the optimal treatment parameters, including time of laser irradiation after dye injection, to achieve selective closure of CNV.

Materials and Methods

Animals

Animals were used in accordance with the Association for Research in Vision and Ophthalmology resolution on the use of animals in research and in accordance with guidelines developed by the Animal Care Committee of the Massachusetts Eye and Ear Infirmary. Cynomolgus monkeys (weighing 3–5 kg) were anesthetized for all procedures using ketamine hydrochloride 20 mg/kg, acepromazine maleate 0.25 mg/kg, or diazepam 1.0 mg/kg, and atropine sulfate 0.125 mg/kg, administered intramuscularly. Supplemental anesthesia of 5 to 6 mg/kg of ketamine hydrochloride was given as needed. Proparacaine HCl (0.5%) was used for topical anesthesia. Pupils were dilated with phenylephrine hydrochloride 2.5% and tropicamide 0.8%. Before PDT, topical atropine sulfate 1% was used to ensure adequate dilation for post-treatment photography. Animals were supplemented with intravenous pentobarbital sodium solution (5 mg/kg) before enucleation and were killed after enucleation with a pentobarbital sodium veterinary euthanasia solution (J.A. Webster, Sterling, MA) given intravenously.

Photography

Fundus photography and fluorescein angiography were performed before and after PDT using a Canon Fundus CF-60Z camera (Lake Success, Long Island, NY). Angiography was performed with 10% sodium fluorescein (0.1 ml/kg) injected intravenously.

Induction of Experimental Choroidal Neovascularization

Choroidal neovascularization was induced by argon green laser burns that were placed in the maculae of cynomolgus monkeys using a modification of Ryan's model.[21,22] The laser parameters were modified to include a 50 μm spot size, 0.1 second duration, and powers ranging from 350 to 450 mW, because these parameters seemed to lead to an improved yield of CNV. Treatment was performed using an argon laser (Coherent Argon Dye Laser #920, Coherent Medical Laser, Palo Alto, CA). The monkeys were followed weekly for 2 to 3 weeks by fundus photography and fluorescein angiography to detect CNV.

Photosensitizer

The liposomal preparation of BPD was provided by Quadra Logic Technologies, Inc (Vancouver, British Columbia, Canada). The dye was preserved in a powder form at 2° to 8 °C and was reconstituted before its use. The dye was brought to room temperature 4 to 28 hours before reconstitution and then diluted in 12 ml of sterile water for injection, giving a dye concentration of 2 mg/ml. The dye (both powder and solution forms) was protected from light at all times. The dye solution volume ranged from

0.5 to 3 ml depending on the dye dose and the weight of the animal. The dye was injected intravenously over 30 seconds, preceded and followed by a 3-ml saline flush. The time interval between dye injection and the initiation of laser irradiation was measured from the end of dye injection.

Photodynamic Therapy

Laser irradiation was applied after the intravenous administration of liposomal BPD verteporfin. Laser light at 692 nm was delivered using an argon/dye laser (Coherent 920, Coherent Medical Laser, Palo Alto, CA), a 200-μm silica optical fiber, and a slit-lamp delivery system (Laserlink, Coherent Medical Laser, Palo Alto, CA). The treatments were performed using a plano fundus contact lens (OGFA, Ocular Instruments, Inc, Bellevue, WA). The treatment spot size at the cornea was set on the Laserlink and confirmed with a precision dial caliper micrometer. The laser power at the focal plane was measured with a power meter (Coherent Fieldmaster, Coherent, Auburn, CA).

Dye Dose and Time of Irradiation After Dye Injection

In the first experiments, PDT was performed using the following dye doses: 1.0, 0.5, 0.375, and 0.25 mg/kg. Light dosimetry was kept constant at an irradiance of 600 mW/cm^2 and fluence of 150 J/cm^2, resulting in a treatment duration of 4′09″ minutes. The spot size also was kept constant at 1250 μm. Irradiation was performed over CNV identified by fluorescein angiography with laser light delivered at various times after dye injection, ranging from 5 to 120 minutes.

Fundus photography was done immediately after treatment, after which the animals were housed in the dark for 24 hours. Fundus photography and fluorescein angiography were performed 24 hours after PDT, followed by enucleation under deep anesthesia and the animals being killed. In some cases, PDT was performed on 2 consecutive days (on separate eyes), and the eyes harvested 24 hours after the second treatment.

In a second set of experiments, selectivity of PDT effect was determined in normal eyes. Because the induction of CNV in this model damages the retina, it is difficult to differentiate the damage secondary to the argon laser burns from the damage related to PDT. To assess the effect on surrounding tissues, PDT, using the same parameters, was applied to normal retina and choroid.

Areas of normal retina and choroid adjacent to the irradiated spots and CNV membranes that were not irradiated served as "dye only" controls. These areas were examined by fluorescein angiography and by histopathology. "Light-only" controls had been investigated in previous experiments, which found that a minimally detectable lesion using light-only required 37 W/cm^2, approximately 100 times the levels used for PDT (unpublished data; [Moulton], presented at the ARVO Annual Meeting, Sarasota, May 1993). Similarly, the irradiances used for PDT are well below levels used for clinical laser photocoagulation (typically 100–1000 W/cm^2).

Histologic Evaluation

All eyes were enucleated under deep anesthesia. The eyes were fixed in modified Karnovsky fixative (pH, 7.4), bisected after 20 minutes, and then replaced in fixative overnight. Tissue then was transferred to 0.1 M cacodylate buffer (pH, 7.4). The eyes were kept at 4 °C at all times. Tissue samples were post-fixed in 2% osmium tetroxide, dehydrated in ethanol, and embedded in Epon, and serially sectioned at 1 μm. For light microscopy, sections were stained with 0.5% toluidine blue and examined with a Zeiss photomicroscope (Axiophot, Oberkochen, Germany). For electron microscopy, thin sections were cut and stained with uranyl acetate in methanol, and Sato lead stains, and examined with Philips #CM 10 transmission electron microscope (Eindhoven, The Netherlands).

Histopathologic Grading

The histologic findings in PDT spots applied to normal retina and choroid were graded from 1 to 5, according to the cumulative effect in various retinal and choroidal levels. Choriocapillaris closure to the retinal pigment epithelium (RPE), and moderate effect on the outer nuclear layer (ONL) were damage that was considered probably acceptable (grades 1–3). More severe ONL damage (grade 4), inner retinal damage (grade 5), or large choroidal vessel damage were considered unacceptable (grade 5).

Results

Angiographic Closure of Choroidal Neovascularization

A total of 69 areas of experimental CNV in 10 monkeys were treated with PDT using liposomal BPD verteporfin.

Table 1. Angiographic Closure of Choroidal Neovascularization

Dye Dose (mg/kg)	No. of CNV Lesions	Time (mins) of Irradiation after Dye Injection	CNV Closure
1	14	5–120	14/14
0.5	11	<60	7/8
		60–80	0/3
0.375	31	<50	17/20
		50–100	5/11
0.25	14	<20	2/2
		20–40	2/4
		≥40	1/8

CNV = choroidal neovascularization.

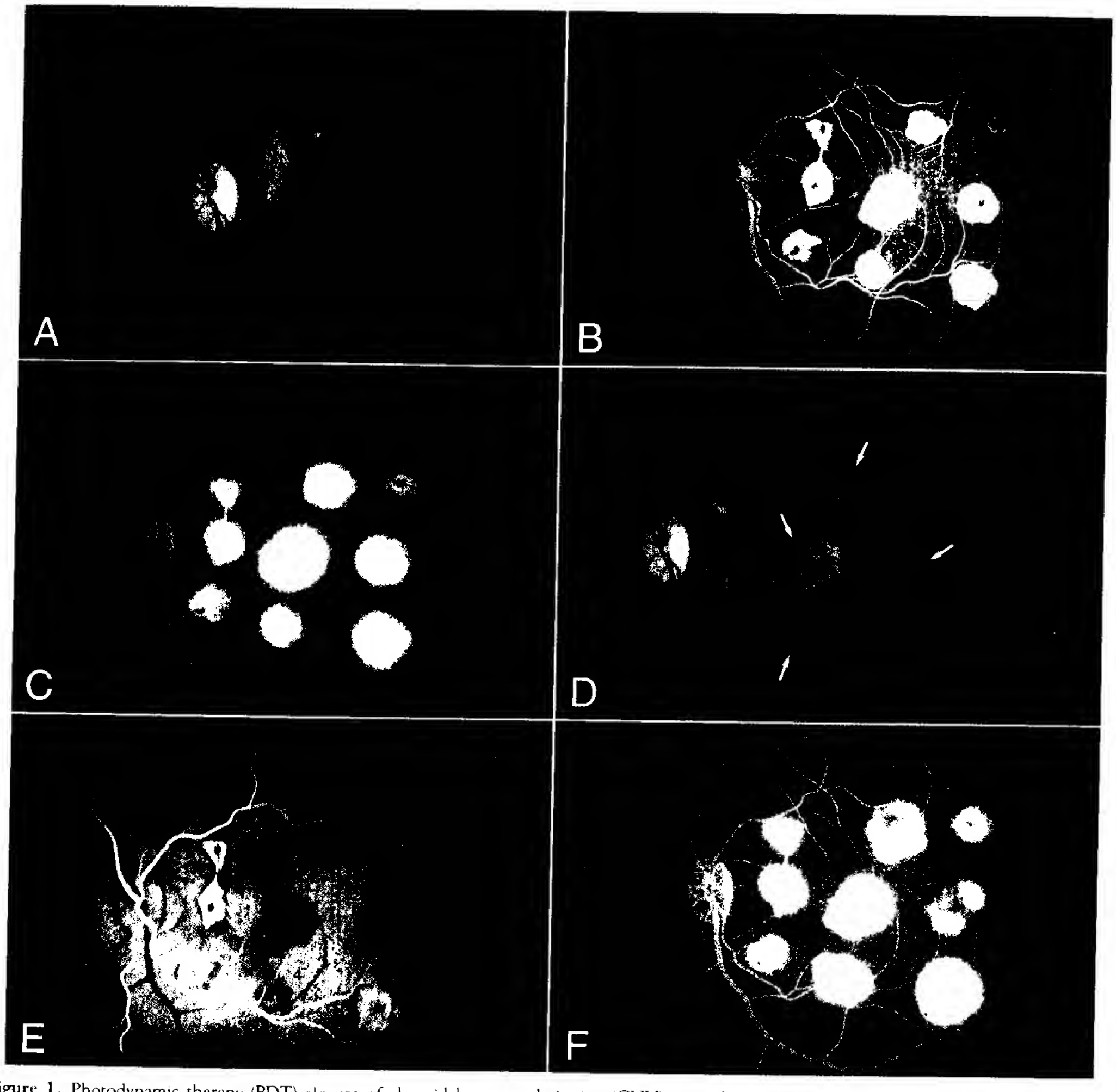

Figure 1. Photodynamic therapy (PDT) closure of choroidal neovascularization (CNV) using 0.5 mg/kg of liposomal benzoporphyrin derivative verteporfin. **A,** color fundus photograph of CNV before PDT. Argon laser burns were placed 1 month previously. **B** and **C,** fluorescein angiogram of CNV before PDT. Areas of CNV show hyperfluorescence in the early frame (1B), with leakage in the later frame (1C). **D,** color fundus photograph 24 hours after PDT using 0.5 mg/kg of liposomal benzoporphyrin derivative verteporfin. There is mild retinal whitening in the treated areas (arrows), compared with the pre-PDT photograph. **E** and **F,** fluorescein angiogram 24 hours after PDT. Lesions were irradiated serially after administration of 0.5 mg/kg of liposomal benzoporphyrin derivative verteporfin using 150 J/cm^2 and 600 mW/cm^2. The time of irradiation after dye injection for lesion 1 was 10 minutes, lesion 2 was 20 minutes, lesion 3 was 40 minutes, and lesion 4 was 50 minutes. Lesions 1, 2, and 3 show hypofluorescence in the early frame (1E), with staining noted in the later frame (1F). The staining developed from the edge of the lesion, typical of PDT lesions. Lesion 4 does not show complete hypofluorescence in the early frame, but has a rim of hypofluorescence in the area that was hyperfluorescent before PDT. The areas of CNV that were not irradiated appear unchanged, with early hyperfluorescence and leakage (three lesions in the nasal macula, and two lesions above and below lesion 2).

Effective CNV closure was demonstrated by fluorescein angiography at all tested dye doses. 1.0, 0.5, 0.375, and 0.25 mg/kg. The lower the dose, the shorter the time interval after dye injection in which laser irradiation produced CNV closure.

The fundus appearance was unchanged immediately after treatment, and only slight deep retinal whitening corresponding to the laser irradiation spot appeared 24 hours later. Choroidal neovascularization closure was determined angiographically at 24 hours by early hy-

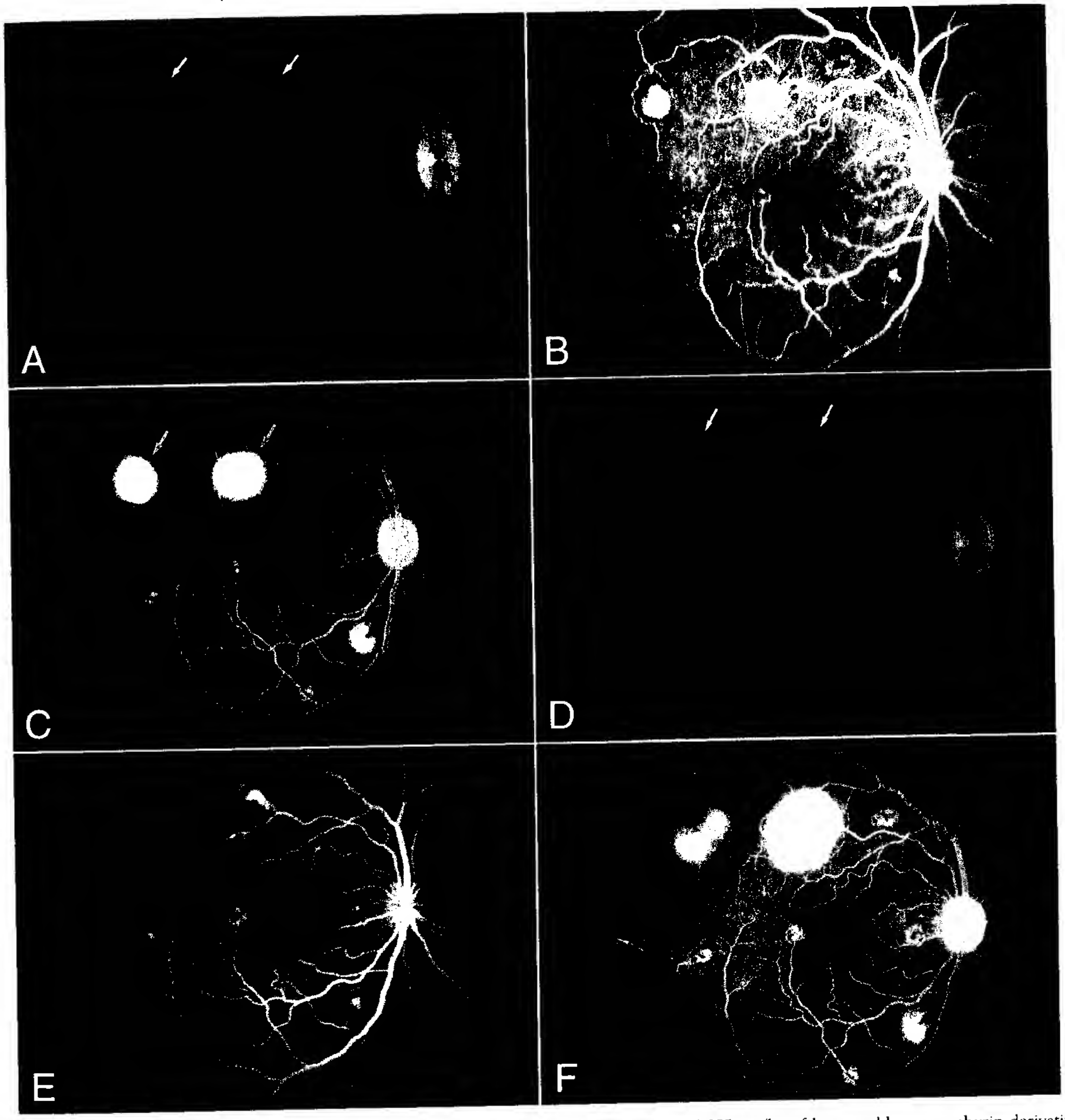

Figure 2. Photodynamic therapy (PDT) closure of choroidal neovascularization (CNV) using 0.375 mg/kg of liposomal benzoporphyrin derivative verteporfin. **A**, color fundus photograph of CNV (arrows) before PDT. Argon laser burns were placed 1 month previously. **B** and **C**, fluorescein angiogram of CNV before PDT. Areas of CNV show hyperfluorescence in the early frame (2B; arrows), with leakage in the later frames (2C; arrows). **D**, color fundus photograph of CNV 24 hours after PDT. As with the higher dye dose, there is mild retinal whitening in the treated areas (arrows) compared with the pre-PDT photograph. **E** and **F**, fluorescein angiogram 24 hours after PDT. Lesions were irradiated serially after administration of 0.375 mg/kg of liposomal benzoporphyrin derivative verteporfin using 150 J/cm^2 and 600 mW/cm^2. The time of irradiation after dye injection for lesion 1 was 20 minutes and for lesion 2 was 25 minutes. Both lesions show central hypofluorescence in the early frame (2E). Staining begins at the periphery of lesion and is seen at the superior and temporal edge of lesion 1 in the early frame with pronounced staining in the late frame (2F). An untreated area of CNV demonstrates early hyperfluorescence and leakage, inferotemporal to the disc.

pofluorescence corresponding to the treated area. As the angiogram progressed, most lesions demonstrated staining starting at the periphery of the lesion. Table 1 summarizes the effect of PDT on CNV, using different dye doses and variable irradiation times after dye injection.

Figure 3. Photomicrographs of a treated choroidal neovascularization (CNV) (**A** and **B**) and an untreated CNV (**C**–**E**). The treated CNV was from an animal killed 24 hours after laser irradiation using 600 mW/cm^2 and 150 J/cm^2, irradiated 20 minutes after injection of 0.375 mg/kg of liposomal benzoporphyrin derivative verteporfin. **A**, light micrograph shows occluded CNV (arrowheads) and choriocapillaris (small arrows); however, the larger choroidal vessels remain patent (large arrow). Note the lack of damage to other cells and structures (bar = 25 μm). **B**, electron micrograph of the same occluded membrane shows two vessels (asterisks) filled with platelets and erythrocytes. The endothelium has been stripped and there is cellular debris around the vessels as well as a viable cell (C). **C**, light micrograph of an untreated CNV shows a full-thickness view. The lesion is filled with pigment-laden cells and small blood vessels (arrowheads). In this region of the lesion, Bruch membrane (arrow) is intact (bar = 25 μm). **D**, a higher magnification light micrograph of the untreated CNV shown in 3C shows several blood vessels (arrowheads), Bruch membrane (arrow), and pigment-laden cells (bar = 10 μm). (Fig 3 *continues.*)

Figure 3 *(continued)* E, electron micrograph of a single blood vessel from the untreated CNV with a small lumen (L), surrounded by hypertrophic endothelial cells (E), a complete basal lamina (arrowheads), pericytes (asterisk), and pigment laden macrophages (bar = 2 μm).

Photodynamic therapy using a dye dose of 1 mg/kg was performed over 14 membranes in 2 monkeys. Laser irradiation was performed at each of the following times after dye injection: 5, 10, 20, 40, 60, 80, 100, and 120 minutes. The CNV closure was induced in all lesions when irradiation was performed 5 to 120 minutes after dye injection.

Photodynamic therapy using a dye dose of 0.5 mg/kg was performed on 11 membranes in 2 monkeys, with laser irradiation at 10, 20, 30, 40, 50, 60, and 80 minutes after dye injection. Photodynamic therapy effect was assessed 24 hours after treatment. Choroidal neovascularization closure was found in 7 of 8 membranes that were irradiated between 10 and 60 minutes after dye injection. Figure 1 demonstrates PDT closure of CNV at this dye dose. The three membranes irradiated at 60 or 80 minutes after dye injection were open on angiography.

Thirty-one areas of CNV in 5 monkeys were treated with PDT using liposomal BPD verteporfin at a dose of 0.375 mg/kg. All treated CNV membranes were assessed angiographically at 24 hours. Figure 2 demonstrates fundus photography and fluorescein angiography of CNV before and after PDT at this dye dose. As indicated in Table 1, 17 of 20 CNV irradiated within 50 minutes after injection demonstrated angiographic closure. Only 5 of 11 membranes irradiated 50 or more minutes after dye injection demonstrated angiographic closure.

A dye dose of 0.25 mg/kg was found to be the threshold dose for PDT using a light dose of 150 J/cm^2 and 600 mW/cm^2. Choroidal neovascularization closure was demonstrated in two of two membranes that were irradiated within 20 minutes after dye injection. Only two of four CNV irradiated between 20 and 40 minutes after dye injection showed closure. Finally, one of eight CNV was irradiated more than 40 minutes after dye injection demonstrated closure.

Histopathologic Findings in Treated Choroidal Neovascularization

Histopathologic confirmation of CNV closure was evident at all tested dye doses: 1.0, 0.5, 0.375, and 0.25 mg/kg. Figure 3 compares the light and electron microscopic findings of PDT treated and untreated CNV. On light microscopy, the closed CNV frequently demonstrated no identifiable vessels, while open vessels could be easily identified in CNV classified as open angiographically. Closed CNV also showed vessels packed with erythrocytes, with occasional extravasated erythrocytes and pockets of fibrin within the tissue as well as in the subretinal space. On electron microscopy, the endothelial cells were missing or severely damaged. Extravasated erythrocytes and occasional leukocytes were noted, and fibrin was visible in the vascular lumina as well as in the surrounding tissue. Stromal cells adjacent to vessels appeared undamaged in most cases, although at the higher doses (0.5, 1.0 mg/kg), some damage was evident. At 0.25 mg/kg, the vessels were

Table 2. Grading Scheme of Photodynamic Therapy Effect on Normal Retina/Choroid

Grade	Damaged Retinal/Choroidal Layers
1	RPE only; or RPE + slight photoreceptor changes + occasional pyknosis in the ONL; with or without choriocapillaris closure
2	Choriocapillaris closure + RPE + photoreceptors + 10%–20% pyknosis in the ONL
3	Choriocapillaris closure + RPE + photoreceptors + ONL pyknosis <50%
4	Choriocapillaris closure + RPE + photoreceptors + ONL pyknosis >50%
5	Choriocapillaris closure + RPE + photoreceptors + ONL pyknosis >50% + choroidal vessel damage or retinal vessel or inner retinal damage

RPE = retinal pigment epithelium; ONL = outer nuclear layer.

packed with erythrocytes, but the endothelial cells seemed to be less damaged.

Treatment Selectivity

Treatment selectivity was investigated by performing PDT in normal retina and choroid using the same dye doses and times of laser irradiation after dye injection. A total of 38 areas of light irradiation preceded by dye injection were placed in normal retina/choroid of 9 monkeys, using the same parameters as were used to treat CNV. The assessment of the damage to the retina and choroid was graded according to the histologic findings at different levels. Table 2 outlines the grading system developed by the authors (MK, JWM, NM, TJF) for this study. The treatment parameters and the degree of effect are summarized in Table 3.

In most cases, the closure of the choriocapillaris in normal choroid followed a similar time course as the closure of CNV. When PDT was performed using dye doses of 0.5, 0.375, and 0.25 mg/kg, the retinal structure was well preserved. In none of the cases were retinal detachment or hemorrhage observed. Reducing the dye dose resulted in more selective closure of the choriocapillaris with minimal damage to the adjacent tissues. The RPE cells were typically damaged at all dye doses as was mild damage to photoreceptor inner and outer segments, ranging from minimal swelling to more pronounced vacuolization and disarray.

Photodynamic therapy using a dye dose of 1 mg/kg led to damage of both inner and outer retina. Areas irradiated within 50 minutes after dye injection demonstrated grade 5 damage, with damage to the inner retina. The sixth lesion was not found on sectioning. Six lesions irradiated 60 minutes or more after dye injection demonstrated grade 4 damage. Two were not found on histopathology.

At a dye dose of 0.5 mg/kg, only the lesion irradiated 5 minutes after dye injection demonstrated damage to the inner retina (grade 5). Lesions irradiated at 20 minutes and later did not affect the inner retina but showed pyknosis in the ONL, vacuolization, and disorganization of the photoreceptors' inner and outer segments, and damage to the RPE (grade 4).

At a dye dose of 0.375 mg/kg, 24 lesions in 3 monkeys were examined (Fig 4). One of the three lesions irradiated 10 minutes after dye injection showed closure of medium-sized choroidal vessels, characterized as grade 5 damage, although ONL pyknosis was minimal. In lesions irradiated within 50 minutes after dye injection, 3 of 15 had grade 1 damage, 4 of 15 had grade 2 damage, 2 of 15 had grade 3 damage, and 3 of 15 had grade 4 damage (Fig 4C). Grade 3 lesions showed some pyknosis in the ONL (<50%), some vacuolization and disorientation of the photoreceptors' inner and outer segments, and damage to the RPE.

A dye dose of 0.25 mg/kg was found to be the threshold dose for induction of choriocapillaris closure. This was achieved with almost no effect on the overlying retina. Five of seven lesions showed grade 1 damage with mild damage to some RPE cells, minimal swelling of photoreceptors, and a few pyknotic nuclei in the ONL (Fig 5). Two lesions irradiated 5 and 10 minutes after dye injection had grade 5 damage.

Dye only control areas of normal retina/choroid showed no effect by fluorescein angiography or histopathologic examination. Although systemic toxicity was not specifically addressed in this study, no adverse systemic effects of dye administration were noted.

Discussion

In this study, we demonstrated effective and selective closure of experimental CNV with PDT using liposomal BPD verteporfin, as a photosensitizer. We previously performed a pilot study to investigate whether PDT using BPD could lead to CNV closure.[14] The current study was designed as a larger, definitive preclinical study to determine the dye dose response and the optimal timing of laser irradiation for both CNV closure and selectivity. Selectivity was assessed in normal retina/choroid by grading the damage to the overlying retina and the subject choroid when the choriocapillaris was closed by PDT. Normal eyes were used to assess selectivity, because the eyes with CNV demonstrated disruption of the inner retina and choroid secondary to the argon laser used to induce the CNV. The effects of PDT were assessed relatively acutely, at 24 hours, with subsequent studies designed to address the long-term effects of PDT. A liposomal preparation was used in this study, because it is a safe, stable preparation, with the potential for clinical use, and it facilitates delivery of dye into the lipoprotein fraction of the blood.[19,20]

To study the selectivity of treatment, we established a grading system describing the histopathologic effects on different levels of normal retina and choroid. When compared with thermal laser lesions, typical of current therapy,[23,24] PDT using liposomal BPD verteporfin appears to be far less destructive. Tso et al graded the thermal damage induced to the retina by xenon arc photocoagulator.[25] Their grading system comprised grades 0 to 3, from no visible change on ophthalmoscopy and light microscopy to full thickness damage. The effects demonstrated by PDT in this study were, for the most part, within grade 1 on Tso's scale. The clinical significance of the observed histopathologic effects of PDT on the retina is unknown.

The pilot study using lipoprotein-delivered BPD gave some guidelines regarding dye and light dosimetry.[14] Effective CNV closure was achieved using 1 to 2 mg/kg of lipoprotein-delivered BPD, using 100 to 150 J/cm^2, and 150 to 600 mW/cm^2, when irradiation was performed between 1 and 81 minutes after dye injection. As the dye dose was reduced from 2 to 1 mg/kg, the fluence required to close the CNV increased from 50 to 100 J/cm^2. This study also demonstrated that higher irradiances were effective without causing apparent thermal damage, thereby providing a more practical treatment duration. However,

Table 3. Photodynamic Therapy Effect on Normal Retina/Choroid

Dye Dose (mg/kg)	Time (mins) of Irradiation after Dye Injection	No. of Lesions	No. of Lesions per Histopathologic Grading				
			1	2	3	4	5
1	<60	6					5
	60–120*	8				6	
0.5	<20	1					1
	20–60	3				3	
0.375	<20	3	1	1			1
	20–50	12†	2	3	2	3	
	50–100	9	3	2	1	3	
0.25	<20	3	1				2
	20–40	4‡	3				
	>40	2‡	1				

* Three lesions irradiated at 40, 100, and 120 minutes were not identified histopathologically.

† Two lesions at 30 and 40 minutes were not identified histopathologically.

‡ Two lesions at 40 and 60 minutes were not identified histopathologically.

a more comprehensive study of dye dose response was needed before considering clinical trials.

The pilot lipoprotein-delivered BPD study also suggested that effectiveness and selectivity of treatment might be greatly affected by the time chosen for irradiation. For instance, irradiation performed within the first 5 minutes after dye injection appeared to cause some damage to retinal vessels and larger choroidal vessels. At this early time point, the dye concentration may be equal in the normal retinal and choroidal vessels and in the CNV. At later time points after dye injection, there may be selective accumulation of dye in the CNV and loss of dye from the normal vessels, as suggested by BPD angiography studies (unpublished data; Miller, presented at the Retina Society Annual Meeting, Williamsburg, VA, 1994; Kramer, presented at the ARVO Annual Meeting, Ft. Lauderdale, FL, 1995).

The starting point for the current study using liposomal BPD verteporfin was a dye dose of 1 mg/kg, fluence of 150 J/cm^2, and irradiance of 600 mW/cm^2, with irradiation performed from 5 to 120 minutes after dye injection. The PDT of CNV using the liposomal preparation at a dye dose of 1 mg/kg was successful, but the damage in normal eyes (grades 5 and 4) was beyond the acceptable range. To reduce the damage to surrounding tissue, we elected to keep the light parameters constant and to study lower dye doses. Reducing the dye dose had two major effects as follows: (1) increased treatment selectivity as assessed by PDT in normal retina and choroid and (2) shortening of the time interval after dye injection in which laser irradiation leads to successful closure of CNV.

The PDT using a dye dose of 0.25 mg/kg was found to be the threshold dose for CNV closure, and the effect on normal retina was minimal (grades 1 and 2) in most cases. However, two of the three areas irradiated early after dye injection (5 or 10 minutes) demonstrated grade 5 effect, with some closure of medium-sized choroidal vessels, although the retinal vessels appeared normal. This damage to medium-sized choroidal vessels with early irradiation was seen at all dye doses tested. With later irradiation times and particularly at higher dye doses, increased pyknosis was seen in the ONL, consistent with transport of dye across the RPE to the photoreceptors, seen in the rabbit localization studies (unpublished data; [Haimovici], presented at the Association for Research in Vision and Ophthalmology Annual Meeting, Sarasota, FL, 1993). Using an above-threshold dye dose of 0.375 mg/kg, we were able to demonstrate a high rate of effective CNV closure when irradiation was performed within the effective time interval after dye injection (<50 minutes). Most treatments in normal retina and choroid using the same dye dose demonstrated choriocapillaris closure with accompanying effects graded 1 to 3 on our scale. This was believed to be acceptable damage, although long-term studies are needed to investigate the histologic recovery after PDT. The extent to which such damage might affect visual function is unknown.

The combined data regarding the effectiveness and selectivity of the treatment lead to the conclusion that the optimal PDT parameters of CNV with a light dose of 150 J/cm^2 and 600 mW/cm^2 are a dye dose of 0.375 mg/kg, with light irradiation performed 20 to 50 minutes after dye injection. Damage to retinal and choroidal vessels was avoided when irradiation was performed more than 20 minutes after dye injection, probably because of the clearance of the dye from the normal retinal and choroidal circulation. Recent angiography studies performed with liposomal BPD verteporfin using a higher dye dose provide indirect evidence to support this assumption (unpublished data, [Kramer], presented at the Association for Research in Vision and Ophthalmology Annual Meeting, Ft Lauderdale, FL, 1995). In these studies using 2 mg/kg of li-

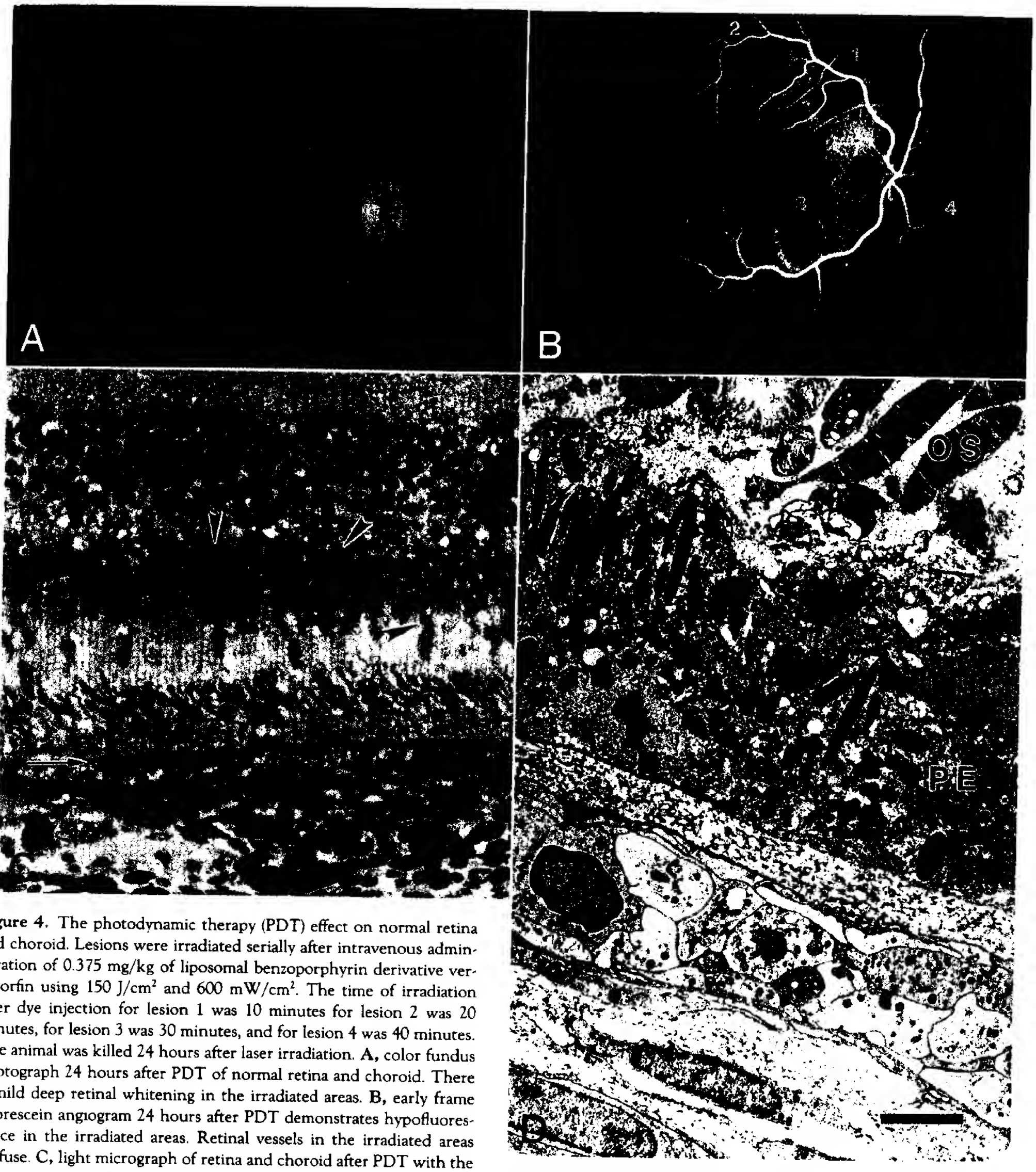

Figure 4. The photodynamic therapy (PDT) effect on normal retina and choroid. Lesions were irradiated serially after intravenous administration of 0.375 mg/kg of liposomal benzoporphyrin derivative verteporfin using 150 J/cm^2 and 600 mW/cm^2. The time of irradiation after dye injection for lesion 1 was 10 minutes for lesion 2 was 20 minutes, for lesion 3 was 30 minutes, and for lesion 4 was 40 minutes. The animal was killed 24 hours after laser irradiation. **A,** color fundus photograph 24 hours after PDT of normal retina and choroid. There is mild deep retinal whitening in the irradiated areas. **B,** early frame fluorescein angiogram 24 hours after PDT demonstrates hypofluorescence in the irradiated areas. Retinal vessels in the irradiated areas perfuse. **C,** light micrograph of retina and choroid after PDT with the parameters given above. The lesion shown (grade 2) was irradiated 20 minutes after dye injection. Note the complete closure of the choriocapillaris and the damaged RPE (Bruch membrane = small arrows). The outer retina shows swelling and some pyknosis of ONL nuclei (arrowheads), and the inner retina shows some swelling and minimal pyknosis (bar = 25 μm). **D,** electron micrograph of the same lesion as C. Note the choriocapillaris closed by platelets (asterisk) and stripped of endothelium. Bruch membrane (arrow) contains fibrin and the RPE is severely damaged (E). Outer segments range from intact to badly swollen (bar = 2 μm).

posomal BPD verteporfin, fluorescence appears in the CNV within the first minute, delineates the CNV well by 5 minutes, and shows marked fluorescence at 30 minutes, with some fluorescence persisting in the CNV out to 2.5 hours with minimal leakage. Fluorescence in the normal choroidal and retinal vessels occurs earlier and fades rapidly: 5 minutes for choroidal vessels and 20 minutes for retinal vessels. Although angiography provides relative

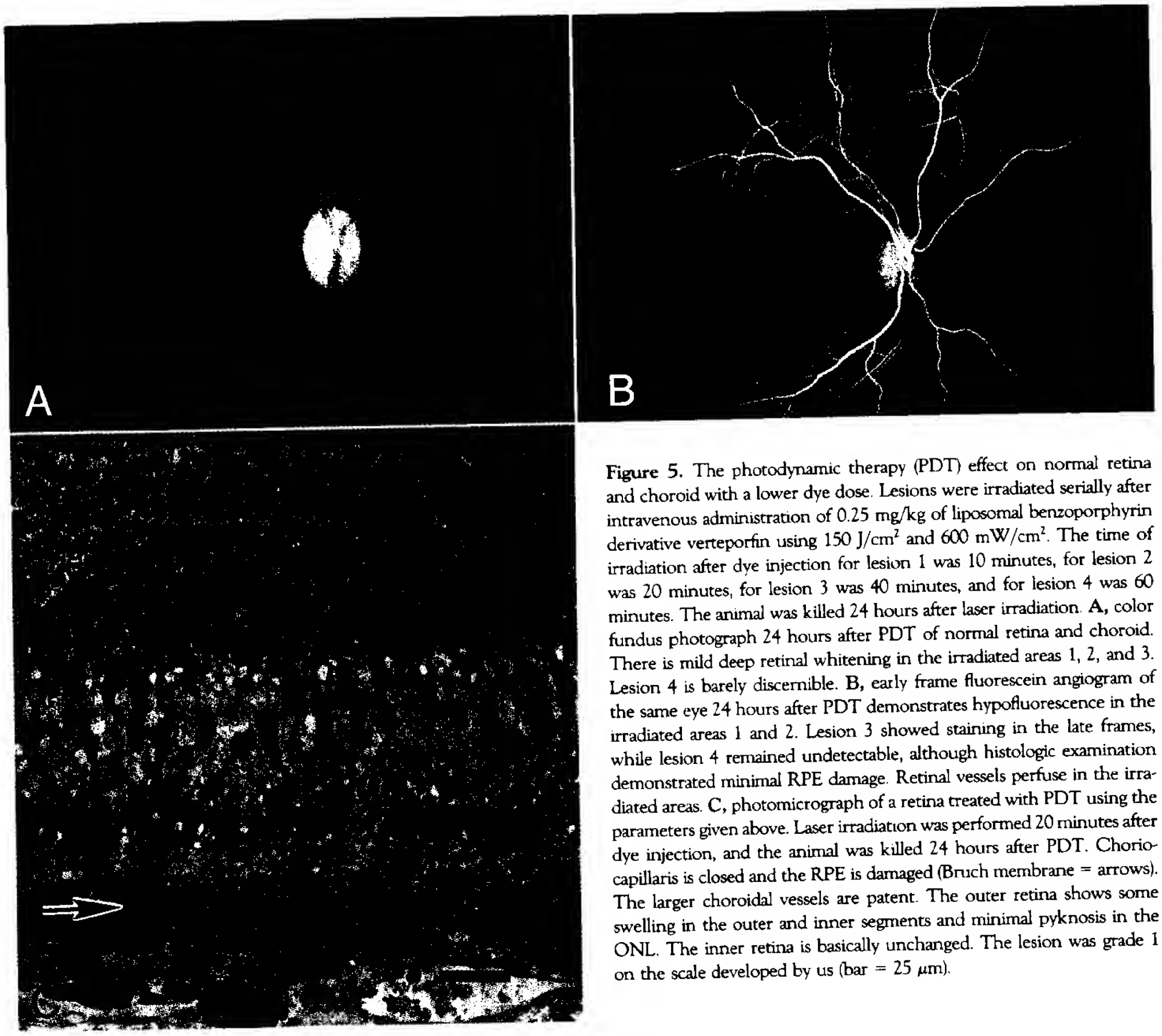

Figure 5. The photodynamic therapy (PDT) effect on normal retina and choroid with a lower dye dose. Lesions were irradiated serially after intravenous administration of 0.25 mg/kg of liposomal benzoporphyrin derivative verteporfin using 150 J/cm^2 and 600 mW/cm^2. The time of irradiation after dye injection for lesion 1 was 10 minutes, for lesion 2 was 20 minutes, for lesion 3 was 40 minutes, and for lesion 4 was 60 minutes. The animal was killed 24 hours after laser irradiation. **A,** color fundus photograph 24 hours after PDT of normal retina and choroid. There is mild deep retinal whitening in the irradiated areas 1, 2, and 3. Lesion 4 is barely discernible. **B,** early frame fluorescein angiogram of the same eye 24 hours after PDT demonstrates hypofluorescence in the irradiated areas 1 and 2. Lesion 3 showed staining in the late frames, while lesion 4 remained undetectable, although histologic examination demonstrated minimal RPE damage. Retinal vessels perfuse in the irradiated areas. **C,** photomicrograph of a retina treated with PDT using the parameters given above. Laser irradiation was performed 20 minutes after dye injection, and the animal was killed 24 hours after PDT. Choriocapillaris is closed and the RPE is damaged (Bruch membrane = arrows). The larger choroidal vessels are patent. The outer retina shows some swelling in the outer and inner segments and minimal pyknosis in the ONL. The inner retina is basically unchanged. The lesion was grade 1 on the scale developed by us (bar = 25 μm).

fluorescence information, it suggests that selective dye accumulation in CNV and selective PDT effect may be achieved 20 to 30 minutes after dye injection.

In conclusion, PDT using liposomal BPD verteporfin is a potential, selective treatment modality that results in direct damage to neovascular tissue with only minimal damage to the retina and choroid. The absorption peak of the dye near 692 nm permits the use of longer wavelength light to treat CNV. The dynamic biodistribution of the dye allows treatment selectivity by adjusting treatment parameters, including the dye dose and time of laser irradiation after dye injection. Using a light dose of 150 J/cm^2 and 600 mW/cm^2 provides a treatment duration of 4′09″ minutes, which is clinically feasible. If the experimental results in this study prove to be safe and effective in humans, PDT using liposomal BPD may be beneficial in the treatment of CNV in age-related macular degeneration. It also is a potential treatment for other forms of ocular neovascularization, such as proliferative diabetic retinopathy, neovascular glaucoma, corneal neovascularization, and ocular tumors.

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60114905	Not Issued	159	01/05/1999	TRANS-SCLERAL CONTROLLED-RELEASE DRUG DELIVERY	GRAGOUDAS, EVANGELOS S.
08209473	5707986	150	03/14/1994	AN ANGIOGRAPHIC METHOD USING GREEN PORPHYRINS IN PRIMATE EYES	GRAGOUDAS, EVANGELOS S.
60291445	Not Issued	020	05/16/2001	IMPLANTED MICROMECHANICAL DELIVERY SYSTEM FOR CHRONIC RETINAL DISEASES	GRAGOUDAS, EVANGELOS S.
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AB Provided are methods and compns. for the photodynamic therapy (PDT) of

ocular conditions characterized by the presence of unwanted **choroidal neovasculature**, for example, neovascular age-related macular degeneration. The selectivity and sensitivity of the PDT method can be enhanced by combining the PDT with an anti-angiogenesis factor, for example, angiostatin or endostatin, or with an apoptosis-modulating factor. Furthermore, the selectivity and sensitivity of the PDT may be further enhanced by coupling a targeting moiety to the photosensitizer so as to target the photosensitizer to **choroidal neovasculature**.

L3 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2002 ACS
2001:[illegible] Document No. 135:[illegible] Photosensitizer targeting for eye disease. Chen, James (Light Sciences Corporation, USA). PCT Int. Appl. WO [illegible] A2 20010712, 20 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG. English. CODEN: PIXXD2. APPLICATION: WO [illegible]-US [illegible]. PRIORITY: US 2000-[illegible]75689 20000111.

AB The invention discloses methods, kits, and instructions to treat neovasculature diseases of the eye through the administration of a targeted photosensitizing agent and subsequent exposure to light of specific wavelength sufficient to photoactivate photosensitizing agent. The photosensitizing agent is bound to a compn. that mediates site specific delivery to a neovasculature target tissue of a therapeutically effective amt. of a photosensitizing agent that is activated by a relatively low fluence rate of light over a prolonged period of time. Diseases treatable under this invention, include: diabetic retinopathy; macular degeneration; and malignant uveal melanomas. Verteporfin is conjugated to a bindable fragment of the LDL antibody demonstrating high affinity to the BD-B of fibronectin for **treatment** of **choroidal neovasculature** lesions.

L3 ANSWER 3 OF 5 SCISEARCH COPYRIGHT 2002 ISI (R)
2000:[illegible] The Genuine Article (R) Number: [illegible]MD. Recent advances in photodynamic therapy. Pandey R K (Reprint). NEW YORK STATE DEPT HLTH, ROSWELL PK CANC INST, PHOTODYNAM THERAPY CTR, BUFFALO, NY 14263 (Reprint). JOURNAL OF PORPHYRINS AND PHTHALOCYANINES (JUN-JUL) 2000, Vol. 4, No. 4, pp. 368-373. Publisher: JOHN WILEY & SONS LTD, BAFFINS LANE CHICHESTER, W SUSSEX PO19 1UD, ENGLAND. ISSN: 1088-4246. Pub. country: USA. Language: English.
'ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS'

AB Clinical results of photodynamic therapy continue to show promise for the **treatment** of various solid malignancies. This paper briefly summarizes the advantages/disadvantages of various so-called 'second-generation' photosensitizers and other medical applications of porphyrin based analogs. Copyright (C) 2000 John Wiley & Sons, Ltd.

L3 ANSWER 4 OF 5 MEDLINE DUPLICATE 1
2001[illegible] Document Number: 20147797. PubMed ID: 11044244. Mechanisms of action of photodynamic therapy with verteporfin for the **treatment** of age-related macular degeneration. Schmidt-Erfurth U; Hasan T. University Eye Hospital, Lubeck, Germany. . SURVEY OF OPHTHALMOLOGY, (2000 Nov-Dec) 45 (3) 195-214. Ref: 97. Journal code: 0421001. ISSN: 0039-6257. Pub. country: United States. Language: English.

AB Age-related macular degeneration, especially the neovascular form of the disease, is the leading cause of blindness in elderly people in developed countries. Thermal photocoagulation is still the preferred **treatment** for choroidal neovascularization that does not involve

the fovea, but it is suitable for only a small number of patients and it can lead to immediate loss of visual acuity. Photodynamic therapy with use of photochemical light activation of verteporfin as a photosensitizer (verteporfin therapy) has been shown to be effective in treating vascularized tumors, and its potential to treat other conditions involving neovascularization has also been suggested. Preclinical and clinical studies have indicated that verteporfin therapy can be used to treat choroidal neovascularization secondary to age-related macular degeneration effectively and safely. Selective occlusion of **choroidal neovasculature** by this therapy causes minimal damage to the neurosensory retina and, therefore, does not induce loss of visual acuity. This benefit allows verteporfin therapy to be used in the large proportion of patients who are not eligible for **treatment** by laser photocoagulation. The mechanistic aspects of the mode of action of light-activated verteporfin are described in this review.

L2 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2001 ACS
1999:788978 Document No. 131:488184 Photodynamic immune modulation (PIM). North, John R.; Hunt, David W. C.; Simmir, Guillermo O.; Ratkay, Leslie G.; Chan, Agnes H.; Lui, Harvey M. D.; Levy, Julia G. (QLT PhotoTherapeutics, Inc., Vancouver, BC, Can.). Proceedings of SPIE-The International Society for Optical Engineering, 3565(Biomedical Optics (BMO '98)), 470-474 (English) 1999. CODEN: PSISDG. ISSN: 1277-786X. Publisher: SPIE-The International Society for Optical Engineering.

AB Photodynamic Therapy (PDT) is accepted for **treatment** of superficial and lumen-containing tumors in regions accessible to activating light and is now known to be effective in closure of **choroidal neovasculature** in Age Related Macular Degeneration. PDT utilizes light absorbing drugs (photosensitizers) that generate the localized formation of reactive oxygen species after light exposure. In a no. of systems, PDT has immunomodulatory effects; Photodynamic Immune Modulation (PIM). Using low- intensity photodynamic regimens applied over a large body surface area, progression of mouse autoimmune disease could be inhibited. Further, this **treatment** strongly inhibited the immune-mediated contact hypersensitivity response to topically applied chem. haptens. Immune modulation appears to result from selective targeting of activated T lymphocytes and redn. in immunostimulation by antigen presenting cells. Psoriasis, an immune-mediated skin condition, exhibits heightened epidermal cell proliferation, epidermal layer thickening and plaque formation at different body sites. In a recent clin. trial, approx. one-third of patients with psoriasis and arthritis symptoms (psoriatic arthritis) displayed a significant clin. improvement in several psoriasis-related parameters after four weekly whole-body PIM **treatments** with verteporfin. The safety profile was favorable. The capacity of PIM to influence other human immune disorders including rheumatoid arthritis is under extensive evaluation.

=> s macular degeneration
L4 18614 MACULAR DEGENERATION

=> s l4 and treatment
L5 3899 L4 AND TREATMENT

=> s l5 and anti-angiostatin
L6 1 L5 AND ANTI-ANGIOSTATIN

=> s l5 and age related
L7 2846 L5 AND AGE RELATED

=> s l7 and photosensitizer
L8 95 L7 AND PHOTOSENSITIZER

=> dup remove l3
PROCESSING COMPLETED FOR L3
L9 59 DUP REMOVE L3 (37 DUPLICATES REMOVED)

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L9 ANSWER 1 OF 59 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2002:426255 Document No.: PREV200200426255. **Treatment** of juxtafoveal and extrafoveal choroidal neovascularization in the era of photodynamic therapy with verteporfin. Jampol, Lee M. (1); Scott, Lance. (1) 645 N. Michigan Ave., Suite 440, Chicago, IL, 60611: l-jampol@northwestern.edu USA. American Journal of Ophthalmology, (July, 2002) Vol. 134, No. 1, pp. 1-101. http://www.ajo.com. print. ISSN: 0002-9394. Language: English.

L9 ANSWER 2 OF 59 RESEARCH COPYRIGHT 2002 ISI (R)
2002:94.1: The Genuine Article (R) Number: 513XT. CME photodynamic therapy for choroidal neovascularization - A review. Woodburn K W; Engelman S J; Blumenkranz M S (Reprint). Stanford Univ, Med Ctr, Dept Ophthalmol, Boswell A 157, Stanford, CA 94305 USA (Reprint ; Stanford Univ, Med Ctr, Dept Ophthalmol, Stanford, CA 94305 USA. RETINA-THE JOURNAL OF RETINAL AND VITREOUS DISEASES AUG 2002 V22 I5. 22, No. 4, pp. 391-405. Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. ISSN: 0275-004X. Pub. country: USA. Language: English.
'ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS'

AB Purpose: To review the biophysical basis and current state of therapy for photodynamic closure of subfoveal choroidal neovascularization in the eye.

Methods: A review of the literature is included, which encompasses the chemical structure, biophysical mechanism of action, range of available agents, status of clinical trials, clinical indications, results of **treatments**, complications, and future directions.

Results: Photodynamic therapy has been shown to be effective in closing both experimental choroidal neovascularization in animal models as well as subfoveal choroidal neovascularization in humans. The therapy results in temporary closure of choroidal new vessels for a period of approximately 1 to 4 weeks. By 1 week, most patients have repermeation or reproliferation of choroidal new vessels resulting in the need for retreatment to achieve continued closure and visual stabilization. Differences exist in the quantum yield, clinical efficiency, and light and sensitizer dose requirements between different classes of agents. Further clinical trials will be required to determine the optimal form of therapy, with verteporfin (Visudyne) as the only currently approved agent. Other agents, including tin etiopurpurin (Purlytin) and motexafin lutetium (Optrin), are currently undergoing phase III, and phase II trials, respectively.

Conclusions: Photodynamic therapy is a promising **treatment** modality shown to be effective in achieving closure and stabilization of vision loss compared with placebo control in eyes with subfoveal choroidal neovascularization.

L9 ANSWER 3 OF 59 MEDLINE DUPLICATE 1
20023494 Document Number: 22116468. PubMed ID: 12116830. Scanning laser system for photodynamic therapy of choroidal neovascularization. Obana Akira; Gohto Yuko. (Department of Ophthalmology and Visual Sciences, Osaka City University Graduate School of Medicine, Osaka City, 545-8585 Japan.. akira-hur@med.osaka-cu.ac.jp) . LASERS IN SURGERY AND MEDICINE, (2002) 30 (5) 377-85. Journal code: 8001105. ISSN: 0196-8092. Pub. country: United States. Language: English.

AB BACKGROUND AND OBJECTIVES: In order to improve selectivity of photodynamic therapy (PDT) to choroidal neovascularization (CNV) associated with **age-related macular degeneration**, a laser scanning technique was applied to perform focal laser irradiation to the retina, and the occlusion effects of a new device to the choriocapillaris were evaluated in primate eyes. STUDY DESIGN/MATERIALS

AND METHODS: The device contains lasers for fundus observation of 785 nm and for PDT of 670 nm, matching the absorption peak of a **photosensitizer**, ATX-S10(Na). The laser irradiated the shape on the retina specified before **treatment** and shut off automatically when the predetermined **treatment** was achieved. The occlusion of the choriocapillaris after PDT was documented by fluorescein and indocyanine green angiography and histology. RESULTS: The area designated for PDT was easily drawn on the touch-screen monitor, and occlusion of the choriocapillaris was achieved precisely in the area pre-selected for **treatment** with 6 J/cm(2) or more of radiance following administration of 3 mg/kg ATX-S10 Na(). CONCLUSIONS: This device is useful for irradiating CNV of any shape, sparing the surrounding retina. Since our previous studies suggested that selective occlusion of CNV would decrease not only the functional disturbance caused by PDT, but also the recurrence of CNV, the present device may allow more effective PDT than the slit-laser system presently used.

L9 ANSWER 4 OF 13 CAPLUS COPYRIGHT 2002 ACS
2002:65914 Document No. 136:147561 Verteporfin infusion-associated pain. Brucker, Natalie; Spaide, Richard F.; Maranan, Leandro; Murray, Jane; Freund, K. Bailey; Slakter, Jason S.; Sorenson, John A.; Yannuzzi, Lawrence A.; Sayer, David R.; Fisher, Yale L. (Vitreous-Retina-Macula Consultants of New York, DuBrinner T. Hertz Retinal Research Center, Manhattan Eye, Ear, and Throat Hospital, New York, NY, USA). American Journal of Ophthalmology, 133(2), 211-214 (English) 2002. CODEN: AJOPAA. ISSN: 0002-9394. Publisher: Elsevier Science Inc..
AB PURPOSE: To det. if oral hydration decreases the incidence of verteporfin infusion-assocd. pain and to find out if other factors play a role in predisposing to this undesired complication in a nonrandomized clin. trial. We prospectively exam. 251 consecutive patients who have been diagnosed with subfoveal choroidal neovascularization secondary to **age-related macular degeneration** and received photodynamic therapy using verteporfin. One hundred twenty-five patients were assigned to receive 500 mL of water orally administered 30 min before beginning the verteporfin infusion, and the remaining 126 consecutive patients were used as controls. Historical and clin. factors in these patients were evaluated for their assocn. with the presence of verteporfin infusion-assocd. pain. RESULTS: Out of 126 patients receiving water before **treatment** 12 (9.6%) experienced verteporfin infusion-assocd. pain. Among the 125 patients who did not get hydration before therapy 29 (23.2%) experienced verteporfin infusion-assocd. pain. There was a statistical difference between the incidence of pain in the two groups ($P = 0.0$). No statistically significant assocn. was evidenced between the presence of pain and participant's baseline characteristics, except for pain on previous administration of verteporfin ($P < 0.01$). Out of 251 total patients 74 (29.5%) developed verteporfin infusion-assocd. pain. Back pain was the most common and occurred in 21 (8.4%) patients, but other sites included leg, groin, chest, buttock, arm, and shoulder pain concurrently or independently. All patients had resoln. of their pain, including chest pain, on cessation of the infusion. CONCLUSIONS: Verteporfin infusion-assocd. pain may be more common than has been previously reported and is not limited to the back area. It appears to be an idiosyncratic reaction to the drug. It does not seem to be prevented by oral hydration before infusion of verteporfin, and no baseline characteristics, other than a history of pain on previous infusion, seem to be predictive of verteporfin infusion-assocd. pain.

L9 ANSWER 5 OF 13 CAPLUS COPYRIGHT 2002 ACS
2002:621994 Document No. 137:161192 Rostaporfin (Miravant Medical Technologies). Hunt, David W. C. (QLT Inc, Vancouver, BC, V5T 4T5, Can.). IDrugs, 5(2), 180-186 (English) 2002. CODEN: IDRUFN. ISSN: 1369-7056. Publisher: Current Drugs Ltd..

AB A review. Pharmacia Corp, under license from Miravant Medical Technologies (formerly PDT Inc), is developing rostaporfin (SnET2, Purlytin), a light-activated cytotoxic drug developed as part of Miravant' PhotoPoint photodynamic therapy PDT) program, for the potential **treatment** of wet **age-related macular degeneration** (AMD). In Jan. 2001, results of phase III trials indicated that rostaporfin had not met the primary efficacy endpoint for the wet form of AMD. At this time, a full review of the data was to be undertaken, and decisions about future development of the drug were to be made after addnl. analyses had been completed. The original licensing agreements included the development of rostaporfin for several ophthalmol., intnl. and urol. indications, and for dermatol. applications including certain skin cancers. However, in August 1999, Miravant reported that it no longer intended to pursue cutaneous metastatic breast cancer (CMBC), in order to focus on AMD. Also in 1999, studies in basal cell carcinoma and AIDS related Kaposi's sarcoma were discontinued because of business considerations. Rostaporfin is activated by red light with a wavelength of 664 nm. It is injected into the patient, where it distributes and selectively binds to plasma lipoproteins, which are present in high concns. by hyperproliferating cells such as cancer cells. After 24 h, the targeted cells are stimulated by red light to activate the compd. This triggers the formation of toxic free radical species that destroy the cells without affecting the surrounding normal tissue. In Jan. 2002, Credit Suisse First Boston Bank. sales for Pharmacia of $40 million in 2003 and $80 million in 2004 [416119], while in the same month, Argus Research predicted peak annual sales for Pharmacia of less than $250 million.

L9 ANSWER 6 OF 58 MEDLINE DUPLICATE 2
2002191196 Document Number: 21914461. PubMed ID: 11836601. Laser targeted photo-occlusion of rat choroidal neovascularization without collateral damage. Nishiwaki Hirotaka; Reimer Kai; Goldberg Morton F; D'Anna Salvatore A; Winsor Stanley A; Grebe Rhonda. Department of Ophthalmology and Visual Sciences, Graduate School of Medicine, Kyoto University, Japan.) PHOTOCHEMISTRY AND PHOTOBIOLOGY, 2002 Feb) 75 (2) 144-54. Journal code: 0376425. ISSN: 0031-8655. Pub. country: United States. Language: English.

AB Laser targeted photo-occlusion (LTO) is a novel method being developed to treat choroidal neovascular membranes (CNV) in **age-related** and other **macular degenerations**. A photosensitive agent, encapsulated in heat-sensitive liposomes, is administered intravenously. A low power laser warms the targeted tissue and releases a bolus of **photosensitizer**. The **photosensitizer** is activated after it clears from the normal choriocapillaris but not from the CNV. Forty-five experimental CNV were induced in seven rats. Five weeks after LTO, complete occlusion was observed by laser targeted angiography (LTA) in 7/9 of treated CNV, and partial occlusion was found in the remaining 2/9. The tissues outside the CNV but within the area treated by LTO showed no flow alteration and no dye leakage. All untreated CNV were patent on LTA at 5 weeks. Light microscopy and electron microscopy confirmed the results in treated and control lesions. Moreover, treated areas next to lesions showed normal photoreceptors, retinal pigment epithelium (RPE), Bruch's membrane and choriocapillaris. These results indicate that LTO may improve current photodynamic therapy by alleviating the need for repeated **treatments** and by avoiding the long-term risks associated with damage to the RPE and occlusion of normal choriocapillaries.

L9 ANSWER 7 OF 58 CAPLUS COPYRIGHT 2002 ACS
2002:617936 Synthesis of receptor-targeted photodynamic therapy compounds for the **treatment** of **age-related macular degeneration** and cancer. Dwyer, Greg T.; Harris, Thomas D.; Edwards, D. S.; Yalamanchili, Padmaja; Kagan, Mikhail; Sanabria, Nahir

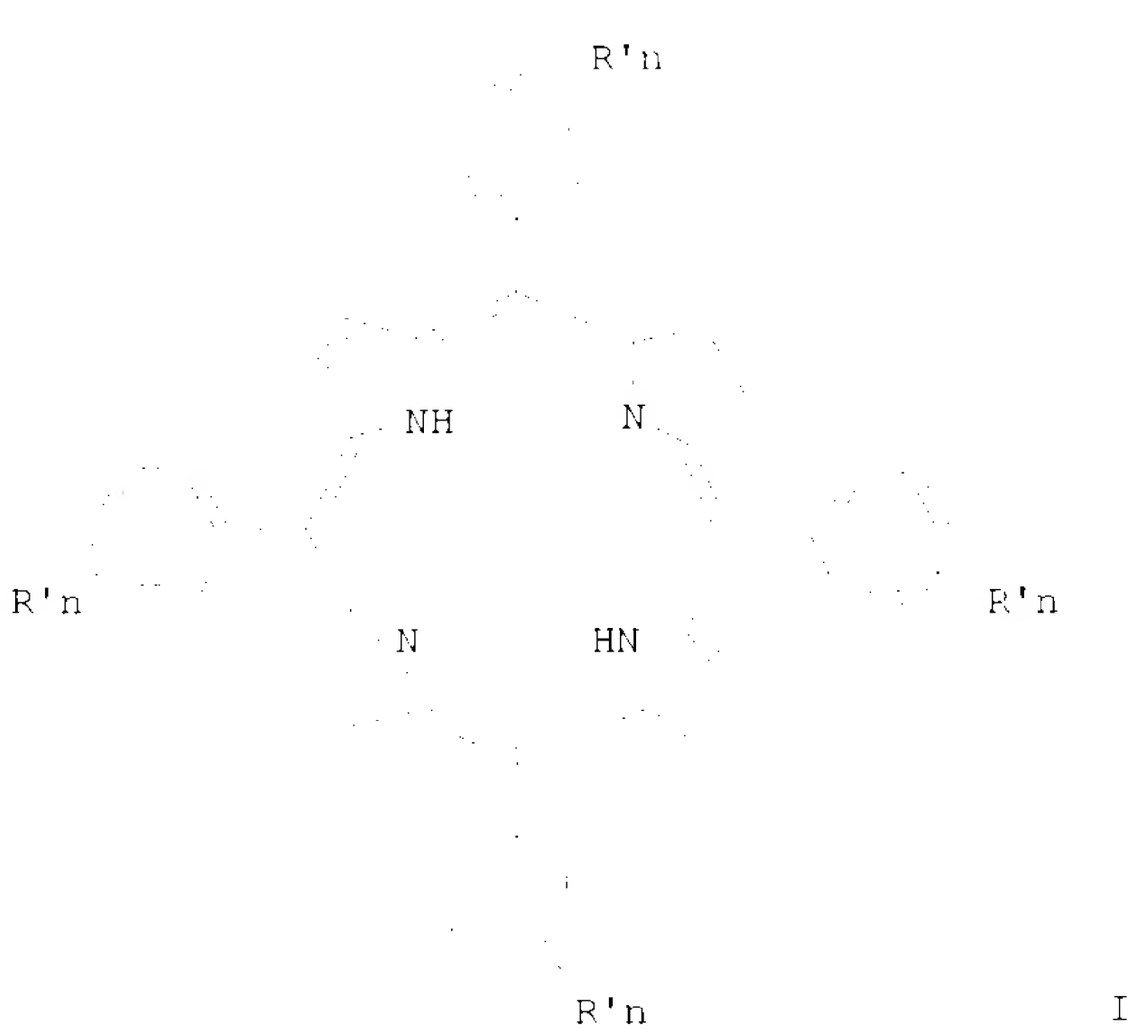
(Discovery Chemistry, Bristol-Myers Squibb Medical Imaging, N. Billerica, MA, 01862, USA). Abstracts of Papers, 224th ACS National Meeting, Boston, MA, United States, August 18-22, 2002, MEDI-092. American Chemical Society: Washington, D. C. (English) 2002. CODEN: 69CZPZ.

AB Photodynamic therapy (PDT) is a modality that employs the combination of light and a photosensitizing drug to generate singlet oxygen and bring about a cytotoxic or modifying effect on target tissue. PDT is currently being employed in the **treatment** of **age-related macular degeneration** AMD), cancer, and other disease states characterized by the presence of cells of high metabolic activity. Verteporfin, trade name VisudyneTM, is currently approved for the **treatment** of AMD. This presentation focuses on the synthesis of verteporfin .alpha. v.beta. 3 receptor antagonist conjugates. Integrin receptor .alpha.v.beta. 3 is selectively expressed in tumor cells and neovasculature related to AMD. Verteporfin .alpha. v.beta. 3 receptor antagonist conjugates would serve as a target-specific means of delivering porphyrin **photosensitizers** to neovasculature and tumor cells. Details of the design, synthesis, and pharmacol. studies of conjugates of verteporfin with quinoline-based .alpha.v.beta. 3 receptor antagonists, such as 1, will be discussed.

L9 ANSWER 8 OF 53 CAPLUS COPYRIGHT 2002 ACS

2001:07672 Document No. 134:242165 Synthesis of poly(alkylene oxide) substituted porphyrin derivs. for use in photodynamic therapy of cancerous and other diseased tissues. Bradley, Paul; Manku, Mehar (Scotia Holdings PLC, UK). PCT Int. Appl. WO 2001006650 A2 20010125, 31 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-GB1010 20010308. PRIORITY: GB 2000-5855 20000310.

GI



I

AB A tetrakis(hydroxyphenyl)chlorin, bacteriochlorin or isobacteriochlorin, derivatized at one or more of the hydroxy groups by addn. reaction with a diisocyanate, diisothiocyanate or isocyanate-isothiocyanate at one isocyanate or isothiocyanate group thereof, the other isocyanate or isothiocyanate group being itself derivatized by addn. reaction with the hydroxy group of an w-alkylated or acylated poly(alkylene oxide) or to a hydroxy group of a link residue itself carrying a residue of such poly alkylene oxide), e.g., I [dashed line = single bond or double bond; R = same or different = OH, O-alkyl, O(C:X)NHANH(C:X)YBnE; X = O, S; Y = O; A = hydrocarbon group contg. 2-41 carbon atoms which may be branched, unbranched, cyclic, acyclic, satd., unsatd., aliph., arom.; B = an optional (-CH2-p-C6H4; p = 1-4; q = 0,1; D = poly(alkylene oxide) with an av. mol. wt. of at least 200 and not more than 40,000; E = alkyl or acyl group contg. 1-12 carbon atoms; n = 1-4], their pharmaceutically acceptable derivs., salts, metal complex, hydrate or solvate, were prepd. for use in photodynamic therapy of cancerous and other diseased tissues. Thus, I [n = 1; meta substitution on all aryl groups; X = O; A = (CH2)6; Y = O; q = 0, D = PEG av. MW = 2000 ; E = Me, III] was prepd. by the coupling of activated mPEG (also prepd.) and 7,8-dihydro-5,10,15,20-tetrakis m-hydroxy phenyl)porphyrin. It showed a singlet oxygen quantum yield of 0.8 +- 0.3 at 0.76 mM/mol/kg.

L9 ANSWER 9 OF 58 CAPLUS COPYRIGHT 2002 ACS
2001:687... Document No. 135:14916 Methods and compositions for treating conditions of the eye. Miller, John W.; Gragoudas, Evangelos S.; Adamis, Anthony P. Massachusetts Eye and Ear Infirmary, USA . PCT Int. Appl. WO 2001058440 A2 20010816, 46 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US41181 20010208. PRIORITY: US 2000-181641 20000210.

AB Provided are methods and compns. for the photodynamic therapy (PDT) of ocular conditions characterized by the presence of unwanted choroidal neovasculature, for example, neovascular **age-related macular degeneration**. The selectivity and sensitivity of the PDT method can be enhanced by combining the PDT with an anti-angiogenesis factor, for example, anecortave or endostatin, or with an apoptosis-modulating factor. Furthermore, the selectivity and sensitivity of the PDT may be further enhanced by coupling a targeting moiety to the **photosensitizer** so as to target the **photosensitizer** to choroidal neovasculature.

L9 ANSWER 10 OF 58 CAPLUS COPYRIGHT 2002 ACS
2001:71911 Document No. 134:107121 Photobleaching of sensitizers used in photodynamic therapy. Bonnett, Raymond; Martinez, Gabriel (Queen Mary, Department of Chemistry, University of London, London, E1 4NS, UK). Tetrahedron, 57(47), 9513-9547 (English) 2001. CODEN: TETRAB. ISSN: 0040-4020. Publisher: Elsevier Science Ltd..

AB A review with refs. on the role of photobleaching in photodynamic therapy, which is an emerging **treatment** for various conditions, particularly for cancer and wet **age-related macular degeneration**. The photobleaching studies in solns. and in cell cultures (in vitro), as well as in vivo photobleaching studies are discussed.

L9 ANSWER 11 OF 58 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 3

2002:10036 The Genuine Article (R) Number: 504RZ. Photosensitisers for the photodynamic therapy of cancer and other diseases. Detty M R Reprint). State University New York Buffalo, Dept Chem, Buffalo, NY 14260 USA (Reprint). EXPERT OPINION ON THERAPEUTIC PATENTS (DEC 2001) Vol. 11, No. 12, pp. 1843-1860. Publisher: ASHLEY PUBLICATIONS LTD. UNITED HOUSE, 3RD FL, 2 ALBERT PLACE FINCHLEY CENTRAL, LONDON N3 1QB, ENGLAND. ISSN: 1354-3776. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Photodynamic therapy is a relatively recent addition to the clinic, primarily for the **treatment** of cancer but also for psoriasis, **age-related macular degeneration** and other diseases. Photodynamic therapy utilises a photosensitiser that targets the disease site to produce a photochemical reaction following delivery of light. The properties of the photosensitiser are critical to the outcome of the technique and numerous classes have been developed in the past decade, including porphyrins and related compounds, chlorins, phthalocyanines, naphthalocyanines, texaphyrins, core-modified porphyrins and various cationic dyes. The potential of this technique is apparent from the extensive number of patents that have been awarded over the past three years.

L9 ANSWER 12 OF 13 CAPLUS COPYRIGHT 2002 ACS
2002:41066 Document No. 136:284256 Verteporfin for **age-related macular degeneration**. Messmer, Karen T.; Abel, Steven R. Richard L Roudebush Veterans Affairs Medical Center, Indianapolis, IN, 46202-2878, USA. Annals of Pharmacotherapy, 36(12), 1993-1998 (English) 2001. CODEN: APHREB. ISSN: 1060-0280. Publisher: Harvey Whitney Books Co..

AB A review. OBJECTIVE: To review the pharmacol., pharmacokinetics, clin. efficacy, adverse effects, drug-drug interactions, and therapeutic issues concerning the use of verteporfin in patients with **age-related macular degeneration** AMD. DATA SOURCES: Published articles and abstrs. in English were identified by MEDLINE (1966-August 2001) searches using the search terms verteporfin, **treatment** of **age-related macular degeneration**, and photodynamic therapy (PDT). Addnl. refs. were identified from the bibliogs. of the retrieved articles. Data were also obtained from approved product labeling. DATA Extn.: The literature was assessed for adequate description of patients, methodol., and outcomes. DATA SYNTHESIS: Verteporfin is a synthetic benzoporphyrin deriv. and a cytotoxic photosensitizing agent, which is one component of PDT. PDT involves administration of verteporfin for injection and nonthermal red light at a wavelength of 689 nm. It is metabolized, to a small extent, to its diacid metabolite by liver and plasma esterases. Information concerning drug interactions is limited. In clin. trials, verteporfin was effective in patients with wet AMD as demonstrated in std. visual acuity scores. Adverse events were related to injection site reactions and visual disturbances. CONCLUSIONS: Verteporfin is a welcome alternative to laser photocoagulation, which can result in damage to the retina and lead to visual loss. Although long-term trials have not been performed in humans, results from monkeys indicate possible improvement in vision following PDT with verteporfin.

L9 ANSWER 13 OF 13 CAPLUS COPYRIGHT 2002 ACS
2001:711216 Document No. 135:46556 Photodynamic therapy with verteporfin for choroidal neovascularization in patients with diabetic retinopathy. Ladd, Byron S.; Solomon, Sharon D.; Bressler, Neil M.; Bressler, Susan E. Retinal Vascular Center, Wilmer Ophthalmological Institute (Department of Ophthalmology), Johns Hopkins University School of Medicine and Hospital, Baltimore, MD, USA). American Journal of Ophthalmology, 132(5), 659-667 (English) 2001. CODEN: AJOPAA. ISSN: 0002-9394. Publisher: Elsevier Science Inc..

AB PURPOSE: To report the use of photodynamic therapy (PDT) with verteporfin

in three patients with choroidal neovascularization (CNV) from **age-related macular degeneration** and underlying diabetic retinopathy. The level of diabetic retinopathy would have excluded these patients from participation in previously reported randomized clin. trials evaluating PDT with verteporfin due to a theoretic concern of damage to the overlying retinal vasculature. DESIGN: Retrospective interventional case series. METHODS: Three patients from a referral practice with at least severe nonproliferative diabetic retinopathy and a history of clin. significant macular edema developed loss of vision from concurrent choroidal neovascularization evaluated with fundus photog. and fluorescein angiog. before and after PDT with verteporfin to identify adverse retinal vascular events. RESULTS: Four eyes in three patients had PDT using verteporfin. Three eyes received two **treatments**. With short follow-up, visual acuity remained stable in two eyes, improved from 20/400 to 20/200 in one eye, and decreased from 20/40 to 20/400 in one eye. Fluorescein angiograms at intervals from 2 wk to 3 mo after PDT showed no damage to the retinal vasculature or progression of the diabetic retinopathy, but did show a decreased area of fluorescein leakage from CNV. One eye that had new subretinal hemorrhage following **treatment** appeared to show new maculopathy on initial evaluation of the post-**treatment** angiogram. Retrospective review suggested that the subretinal hemorrhage provided increased contrast to more easily visualize maculopathy that was present before the PDT. CONCLUSIONS: Three patients with diabetic retinopathy undergoing a total of seven PDT **treatments** with verteporfin in four eyes had no new retinal vascular abnormalities develop. No other atypical responses of CNV to PDT were noted except new subretinal hemorrhage, possibly increased contrast of the overlying vasculature, which gave the false impression of the development of new maculopathy in one eye. Patients with diabetic retinopathy who have concurrent CNV to which PDT with verteporfin is recommended should be cautioned regarding the theor. concerns of harming the retinal vasculature. Periodic surveillance for such concerns seems warranted until more experience is obtained.

L9 ANSWER 14 OF 55 CABLUS COPYRIGHT 2012 ACS

2001:314616 Document No. 135:177264 Verteporfin therapy of subfoveal choroidal neovascularization in **age-related macular degeneration**: 2-year results of a randomized clinical trial including lesions with occult with no classic choroidal neovascularization - verteporfin in photodynamic therapy. Report 2. Verteporfin In Photodynamic Therapy Study Group, Novartis Ophthalmics AG, Bulach, Switz. . American Journal of Ophthalmology, 131(5), 541-560 English. 2001. CODEN: AJOPAA. ISSN: 0002-9394. Publisher: Elsevier Science Inc..

AB It was detd. if photodynamic therapy with verteporfin can safely reduce the risk of vision loss in patients with subfoveal choroidal neovascularization caused by **age-related macular degeneration** who were identified with a lesion comprised of occult with no classic choroidal neovascularization, or with presumed early onset classic choroidal neovascularization with good visual acuity letter score. Verteporfin (6 mg/m2 of body surface area) or placebo was administered by i.v. infusion of 30 mL over 10 min. 15 Min after the start of the infusion, a laser light at 689 nm delivering 50 J/m2 by application of an intensity of 600 mW/cm2 over 83 s using a spot size with a diam. 1000 .mu.m larger than the greatest linear dimension of the choroidal neovascularization lesion in the retina. Verteporfin-treated patients received 4 **treatments** over the 24 mo of follow up. By the month 24 exam., 54% of the verteporfin-treated patients compared to 67% of placebo-treated patients lost at least 15 letters and 30 vs. 47% lost at least 30 letters. In the subgroup of patients with a baseline lesion comprn. identified as occult choroidal neovascularization with no classic choroidal vascularization the results were 55 vs. 68% (loss of 15 letters) and 29 vs. 47% (loss of 30 letters), resp. Results of the

subgroup suggested that the **treatment** benefit was greater for patients with either smaller lesions or lower levels of visual acuity at baseline. A severe decrease of vision (at least 10 letters compared with the visual acuity just before the **treatment**) was found in 4.4 % of verteporfin-treated patients within 7 days after **treatment**, judged to be the result of the development of subretinal pigment epithelial blood, marked subretinal fluid associated with choroidal hypofluorescence, or no obvious cause.

L9 ANSWER 15 OF 56 SCISEARCH COPYRIGHT 2002 ISI (R)
2001:[illegible] The Genuine Article (R) Number: 481DF. Photodynamic therapy of experimental choroidal neovascularization with a hydrophilic **photosensitizer** - Mono-L-aspartyl chlorin e6. More Reprints
Yoneya S; Anzai K; Nakasawa J; Kodeyama T; Peyman G A; Moshfeghi D M. Saitama Med Sch, Dept Ophthalmol, 38 Morohongo, Moroyama, Saitama 3500495, Japan (Reprint); Saitama Med Sch, Dept Ophthalmol, Moroyama, Saitama 350-04, Japan; Tulane Univ, Hlth Sci Ctr, Dept Ophthalmol, New Orleans, LA 70112 USA. RETINA-THE JOURNAL OF RETINAL AND VITREOUS DISEASES (SEP 2001) Vol. 21, No. 5, pp. 448-457. Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. ISSN: 0275-004X. Pub. country: Japan; USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Purpose: To demonstrate the selective localization of the hydrophilic **photosensitizer** mono-L-aspartyl chlorin e6 (NPe6) in experimental choroidal neovascularization in nonhuman primate eyes.

Methods: Sixty-seven experimental choroidal neovascular lesions (CNV) were created in the fundi of Macaca monkeys using the modified Ryan's model and documented by fluorescein and indocyanine green angiography. To determine the biodistribution of NPe6 and the optimal timing of laser irradiation after dye administration, NPe6 angiography and fluorescence microscopy with NPe6 were performed. Photodynamic therapy (PDT) was performed at various dye doses (1.0-1.5 mg/kg) and laser fluences (7.1-21.5 J/cm2) in the CNV and on 11 areas of normal retina and choroid. **Treatment** outcomes were assessed by fluorescein and indocyanine green angiography and confirmed by light and electron microscopy.

Results: NPe6 fluorescence microscopy demonstrated intense fluorescence of CNV and retinal pigment epithelial cells. Choroidal vessel walls and outer retina adjacent to CNV fluoresced moderately; retinal vessel walls and nonencapillaries had trace fluorescence. The fluorescence of CNV lesions on fluorescein angiography became stronger than that of retinal vessels 5-10 minutes after dye injection. Choroidal neovascular lesion closure was achieved with NPe6 PDT without significant damage to the sensory retina. Histology demonstrated necrosis of CNV endothelial cells with minimal damage to surrounding tissues.

Conclusions: NPe6 PDT selectively localizes in experimental CNV in nonhuman primates, resulting in occlusion of CNV with sparing of the neurosensory retina.

L9 ANSWER 16 OF 56 SCISEARCH COPYRIGHT 2002 ISI (R)
2001:[illegible] The Genuine Article (R) Number: 481DF. Retreatment effect of Npe6 photodynamic therapy in the normal primate macula. Nakashizuka T; Mori K; Hayashi H; Ando A; Kondo M; Yoneya S; Moshfeghi D M; Peyman G A (Reprint). Tulane Univ, Hlth Sci Ctr, Dept Ophthalmol, 1430 Tulane Ave SL-69, New Orleans, LA 70112 USA (Reprint); Tulane Univ, Hlth Sci Ctr, Dept Ophthalmol, New Orleans, LA 70112 USA; Tokushima Gen Hosp, Dept Ophthalmol, Tokyo, Japan; Univ Tennessee, Dept Ophthalmol, Memphis, TN 38163 USA. RETINA-THE JOURNAL OF RETINAL AND VITREOUS DISEASES (SEP 2001) Vol. 21, No. 5, pp. 493-499. Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. ISSN: 0275-004X. Pub. country: USA; Japan. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Purpose: To evaluate the safety and efficacy of repeated photodynamic

therapy (PDT) with mono-L-aspartyl chlorin e6 (NPe6) on normal primate fovea and choroid.

Methods: Macaca fascata monkeys were used as experimental subjects. Mono-L-aspartyl chlorin e6 at a dose of 2 mg/kg was administered by intravenous infusion. Laser irradiation was applied within 5 minutes using a 664-nm diode laser at a power output of 595 mW (75 MW/cm(2)), spot size of 1,000 mum, and time of 10 seconds. This resulted in a fluence of 7.5 J/cm(2). Three consecutive PDT **treatments** at 2-week intervals were applied over the center of the fovea and posterior fundus near the arcade vessels of each eye. The animals were killed and the eyes were enucleated for histologic study 2 weeks after the last **treatment**.

Results: Limited changes could be observed in the sensory retina under light microscopy. Photoreceptor cells and outer segments were not damaged, even after repeated PDT. Proliferation and duplication of the retinal pigment epithelial cells were common findings. A plaque of fibrous tissue was present, interwoven with retinal pigment epithelial cells in eyes that received repeated PDT. The retinal vessels remained patent even after three sessions of PDT. However, occlusion of the choriocapillaris and the large choroidal vessels was observed after repeated PDT **treatment**.

Conclusion: Repeated PDT of healthy nonhuman primate fundi using a hydrophilic **photosensitizer** (NPe6) shows preservation of the neurosensory retina components and architecture with damage confined to the retinal pigment epithelium and choriocapillaris.

L9 ANSWER 17 OF 19 SCISEARCH COPYRIGHT 2002 ISI (R)
2001:569615 The Genuine Article (R) Number: 442DF. Clinicopathologic studies of **age-related macular degeneration** with classic subfoveal choroidal neovascularization treated with photodynamic therapy. Ghazi N G; Tabandeh H; De la Cruz Z C; Green W R (Reprint). Johns Hopkins Univ Hosp, Eye Pathol Lab, Maumenee 427, 600 N Wolfe St, Baltimore, MD 21287 USA (Reprint); Johns Hopkins Med Inst, W Maumenee Green Eye Pathol Lab, Baltimore, MD 21205 USA; Johns Hopkins Med Inst, Dept Pathol, Baltimore, MD 21205 USA. RETINA-THE JOURNAL OF RETINAL AND VITREOUS DISEASES (SEP-OCT 2001) Vol. 21, No. 5, pp. 473-486. Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. ISSN: 0275-004X. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background: Photodynamic therapy (PDT) is a relatively new modality that is currently under clinical and experimental evaluation for **treatment** of subfoveal choroidal neovascularization (CNV). The authors report the case of an 81-year-old woman who underwent verteporfin-mediated PDT for classic subfoveal CNV. Fluorescein angiography performed 2 weeks after **treatment** disclosed reduction of the initial area of neovascularization and leakage by approximately 50%. Three weeks after PDT, however, the area of leakage was almost the same size as that before **treatment**. The patient underwent submacular membranectomy almost 4 weeks after **treatment**. The authors describe the ultrastructural vascular changes after PDT and a clinicopathologic study of classic CNV.

Methods: The submacular membrane was studied by light and electron microscopy and immunohistochemical techniques.

Results: Ultrastructural examination of the peripheral vessels showed evidence of endothelial cell degeneration with platelet aggregation and thrombus formation. Occasional occluded vessels were surrounded by macrophages, a phenomenon previously reported to describe the process of resorption of such blood vessels. The vessels in the center of the membrane were unremarkable.

Conclusion: Photodynamic therapy causes endothelial cell damage, thrombus formation, and vascular occlusion of classic CNV in **age-related macular degeneration**.

L9 ANSWER 18 OF 58 CAPLUS COPYRIGHT 2002 ACS
2001:686350 Document No. 136:163809 Photodynamic therapy and transpupillary thermotherapy for neovascular maculopathy. Hayashi, Atsushi (Osaka Univ. Graduate Sch. Med., Suita, Osaka, 565-0871, Japan). Reza Kenkyu, 29(7), 493-497 (Japanese) 2001. CODEN: REKEDA. ISSN: 0387-0200. Publisher: Reza Kenkyukai.

AB A review. Photodynamic therapy (PDT) and transpupillary thermotherapy (TTT) are new **treatment** modalities for neovascular maculopathies such as associd. with **age-related macular degeneration**. PDT consists of two steps. First, photosensitizing dyes are introduced i.v. and taken up by neovascular tissues. Then, laser of specific wavelength to activate the photosensitizing dye is applied to the neovascular tissues to occlude the vessels. Verteporfin and other photosensitizing dyes are introduced and recent results of PDT are described. TTT is another new technique for treating malignant melanoma and subretinal neovascular tissues. IR laser is applied to subretinal tissues to increase the temp. of the tissue up to 45-47 degrees Recent results of TTT are described. Although PDT and TTT still have problems and limitations, we can treat more patients with neovascular maculopathy by these new therapies.

L9 ANSWER 19 OF 58 MEDLINE DUPLICATE 4
2001312769 Document Number: 21494949. PubMed ID: 11395116. [Photodynamic therapy in choroidal new vessels]. Therapie photodynamique des neovaisseaux choroidiens. Soubrane G. Clinique Ophtalmologique Universitaire, 40, avenue de Verdun, 94010 Creteil, France. JOURNAL FRANCAIS D OPHTALMOLOGIE, (2001 Apr) 24 (4) 411-8. Ref: 15. Journal code: 7911115. ISSN: 0181-5512. Pub. Country: France. Language: French.

AB Photodynamic therapy (PDT) is a new approach for subfoveal choroidal new vessels (CNV) in **age-related macular degeneration** (ARM) and myopia, currently being evaluated in clinical trials. PDT is a two-step procedure: the intravenous perfusion of a **photosensitizer** is followed by light irradiation at the adapted wavelength. Verteporfin, the **photosensitizer** under investigation, has a maximum absorption at 689nm. Phase I and II studies determined the settings necessary to obtain optimal effects in humans with Verteporfin in the phase III study. It has been shown that this **treatment** is efficient and preserves initial visual acuity in 67% of Verteporfin-treated ARM eyes vs 19% of placebo-treated ARM eyes at 1 year. Fluorescein angiographic follow-up found a photo-occlusion of the CNV 14 days after **treatment** application followed by a partial reperfusion or reproliferation of the CNV at 3 months, resulting in the need for repeated **treatments**. Two-year results of the Phase III randomized clinical trial are awaited.

L9 ANSWER 20 OF 58 CAPLUS COPYRIGHT 2002 ACS
2001:470796 Document No. 135:165725 Motexafin lutetium. Pharmacyclics). Young, Pollen K. F. College of Pharmacy, Dalhousie University, Halifax, NS, B3H 3J9, Can.). IDrugs, 4(5), 551-559 (English) 2001. CODEN: IDRUFN. ISSN: 1369-7056. Publisher: Current Drugs Ltd..

AB A review with 96 refs. Pharmacyclics is developing the **photosensitizer**, motexafin lutetium as Antrin for the potential **treatment** of restenosis and atherosclerotic plaques, Lutrin for the possible **treatment** of cancer, and Optrin for the possible **treatment** of **age-related macular degeneration** (AMD). Antrin: A phase II multicenter, randomized, controlled study involving 579 peripheral artery disease (PAD) patients is being conducted in the US. It is designed to evaluate Antrin photoangioplasty as a primary **treatment** for PAD and for the prevention of restenosis following balloon angioplasty [341341,347151]. Lutrin: Lutrin is being developed for the possible **treatment** of a no. of cancers [34-919]. In July 1997, the compd. entered phase II trials for breast cancer [323952,323929]. In August 2000, the compd. was

undergoing a phase IIb trial for advanced refractory breast cancer [?30?34]. Optrin: In May 2000, Pharmacyclics reported preliminary results from an ongoing phase II dose-ranging study with Optrin for the photodynamic therapy of patients with AMD [365?74].

L9 ANSWER 21 OF 58 CAPLUS COPYRIGHT 2001 ACS
2001:114?76 Document No. 135:17?762 Verteporfin: A milestone in ophthalmology and photodynamic therapy. Mellish, Kirstie J.; Brown, Stanley B. (Centre for Photobiology and Photodynamic Therapy, University of Leeds, UK). Expert Opinion on Pharmacotherapy, 2(2), 351-361 (English) 2001. CODEN: EOPHF7. ISSN: 1465-66??. Publisher: Ashley Publications Ltd..

AB A review with 54 refs. During the past year, a **photosensitizer** named benzoporphyrin deriv. (BPD) has been approved in 26 countries under the generic name verteporfin. Visudyne, Novartis , for the **treatment** of patients with a certain type of the wet form of **age-related macular degeneration** (AMD) by photodynamic therapy (PDT). AMD is the leading cause of blindness in the developed world, with approx. half a million new cases of the wet form per yr. The approval of Visudyne therapy represents a major milestone in ophthalmol. since AMD was previously untreatable by any modality which would preserve existing vision. It was also a milestone in the development of PDT, not only because it represented the first breakthrough in the use of PDT to treat an otherwise untreatable condition, but also because it represented the first mass market for a PDT **treatment** where prospects of a substantial financial return on many years of investment appear to be likely. In this article, we look at the background to the development of BPD, primarily for its use in AMD, but also in other applications.

L9 ANSWER ? OF 58 MEDLINE DUPLICATE 5
20016?104 Document Number: ??????6?. PubMed ID: 11733?41. Bcl-... increases emptying of endoplasmic reticulum Ca2+ stores during photodynamic therapy-induced apoptosis. Granville D J; Ruehlmann D O; Choy J C; Carthy B A; Hunt D W; van Breemen ?; McManus B M. (UBC McDonald Research Laboratories and the iCAPTURE Centre, St. Paul's Hospital and University of British Columbia, Vancouver, BC, Canada.) CELL CALCIUM, (2001 Nov) 30 (5) 344-?. Journal code: ?06226. ISSN: 0143-4160. Pub. country: Scotland: United Kingdom. Language: English.

AB Photodynamic therapy (PDT) is clinically approved for the **treatment** of several types of cancer as well as **age-related macular degeneration**, the leading cause of blindness in the elderly. PDT using the **photosensitizer** verteporfin has been previously shown to induce rapid apoptosis via a mitochondrial-caspase activation pathway. The impact of PDT on other cellular organelles such as the endoplasmic reticulum (ER) is undefined. The effect of PDT on intracellular Ca2+ ([Ca2+]i) in control and Bcl-2-overexpressing HeLa cells was assessed. A greater [Ca2+]i transient was observed for Bcl-2 overexpressing cells in response to PDT. The PDT-induced Ca2+ release was due to the emptying of Ca2+ from ER and possibly mitochondrial stores and was not due to an influx of Ca2+ from the medium. For Bcl-2-transfected cells, the release of Ca2+ was incomplete as determined by a further [Ca2+]i transient produced by the addition of the Ca2+ ionophore ionomycin after PDT. Furthermore, extrusion of Ca2+ was not hindered while ER-mediated sequestration of Ca2+ was impaired after PDT. Impairment of ER-mediated sequestration of Ca2+ may be due to the immediate caspase-independent depletion of sarco/endoplasmic reticulum Ca2+ ATPase-2 (SERCA2) that occurred in response to PDT in both HeLa Neo and Bcl-2 overexpressed HeLa cells. In summary, PDT induced the rapid degradation of SERCA2 and release of ER and mitochondrial Ca2+ stores. Although overexpression of Bcl-2 did not protect against SERCA2 degradation, it may influence the release of Ca2+ from ER and mitochondrial stores in PDT-treated cells.

L9 ANSWER 23 OF 59 MEDLINE DUPLICATE 6
2001146431 Document Number: 21036572. PubMed ID: 11193207. Photodynamic therapy: shedding light on the biochemical pathways regulating porphyrin-mediated cell death. Granville D J; McManus B M; Hunt D W. (QLT Inc., Vancouver, Canada.. dgranville@qltinc.com) . HISTOLOGY AND HISTOPATHOLOGY, (2001 Jan) 16 (1) 309-17. Ref: 91. Journal code: 8609357. ISSN: 0213-3911. Pub. country: Spain. Language: English.

AB Photodynamic therapy (PDT) is a clinically approved **treatment** for the ocular condition **age-related macular degeneration**, and certain types of cancer. PDT is also under investigation for other ocular, as well as, immune-mediated and cardiovascular indications. PDT is a two step procedure. In the first step, the **photosensitizer**, usually a porphyrin derivative, is administered and taken up by cells. The second step involves activation of the **photosensitizer** with a specific wavelength of visible light. Exposure to light of an activating wavelength generates reactive oxygen species within cells containing **photosensitizer**. PDT with porphyrin **photosensitizers** induces rapid apoptotic cell death, an event which may be attributed to the close association of these compounds with mitochondria. Thus, PDT is an attractive method to treat ailments such as cancer, viral infections, autoimmune disorders and certain cardiovascular diseases in which the apoptotic program may be compromised. The present review examines the cellular events triggered at lethal and sublethal PDT doses and their relationship to the subsequent effects exerted upon cells.

L9 ANSWER 24 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
2002392084 EMBASE Photodynamic therapy with verteporfin: A new **treatment** in ophthalmology. Michels S.; Schmidt-Erfurth U., Dr. U. Schmidt-Erfurth, University Eye Hospital Lübeck, Ratzeburger Allee 160, D-23538 Lübeck, Germany. ulrich@schmidterfurth@oph.mu-luebeck.de. Seminars in Ophthalmology 16 4 221-230 2001.
Refs: 43.
ISSN: 0882-0538. CODEN: SEOPE7. Pub. Country: Netherlands. Language: English. Summary Language: English.

AB Photodynamic therapy (PDT) with verteporfin is a new **treatment** modality in ophthalmology that has previously shown its effectiveness in **treatment** of a variety of neoplastic pathologies. In this therapeutic approach, the **photosensitizer** verteporfin is activated by non-thermal laser light to obtain closure of neovascular structures. Preclinical and clinical studies have indicated that PDT is a safe, selective, and effective **treatment** for choroidal neovascularization in **age related macular degeneration**. No significant damage to the neurosensory retina was found, which explains why PDT does not cause loss of visual acuity and may be used in a larger population than laser photocoagulation. This review summarizes the mechanisms of action of PDT, and the results of preclinical and clinical studies in ophthalmology.

L9 ANSWER 25 OF 59 CAPLUS COPYRIGHT 2001 ACS
2001:546764 Document No. 135:109316 Porphyrin-based sensitizers in the detection and **treatment** of cancer: recent progress. Vicente, M. G. H. Departments of Chemistry and Neurological Surgery, University of California, Davis, CA, 95616, USA). Current Medicinal Chemistry: Anti-Cancer Agents, 1(1), 175-194 (English) 2001. CODEN: CMCACH. ISSN: 1568-0118. Publisher: Bentham Science Publishers Ltd..

AB A review with 291 refs. It has been known for some time that porphyrins and related compds. have the ability to selectively accumulate in tumor tissues, and to persist there for long periods of time. This property, along with the well-described photophys. and photosensitizing properties of porphyrin-type mols., has led to their potential use as adjuvants and sensitizers in a variety of medical applications, such as in photodynamic

therapy (PDT), boron neutron capture therapy (BNCT), radiation therapy (RT) and in magnetic resonance imaging (MRI). Both PDT and BNCT are binary cancer therapies that involve activation of tissue-localized sensitizers with either light (in PDT) or low-energy neutrons (in BNCT). In both of these therapeutic methodologies, local tumor control with minimal side effects relative to other forms of cancer **treatment** (surgery, radiotherapy, chemotherapy) can be achieved. Porphyrins constitute a major class of pharmacol. agents currently under investigation. Photofrin, a porphyrin deriv., has been approved in the USA as a PDT drug by the U.S. Food and Drug Administration (FDA), and also in Japan, Canada and in eleven European countries. Recently, the FDA approved Visudyne, another porphyrin deriv. for the PDT **treatment** of the "wet-form" of **age-related macular degeneration**. In addn. to cancer **treatment** porphyrins are also under investigation for application in the **treatment** of a variety of other diseases.

L9 ANSWER 16 OF 44 SCIFINDER COPYRIGHT 2002 ACS (R) DUPLICATE
2001:166718 The Genuine Article (R) Number: 410BT. Texaphyrins: a new approach to drug development. Mody T D (Reprint); Sessler J L. Pharmacyclics Inc, 995 E Arques Ave, Sunnyvale, CA 94085 USA (Reprint); Pharmacyclics Inc, Sunnyvale, CA 94085 USA; Univ Texas, Dept Chem & Biochem, Austin, TX 78712 USA. JOURNAL OF PORPHYRINS AND PHTHALOCYANINES (FEB 2001) Vol. 5, No. 2, pp. 164-142. Publisher: JOHN WILEY & SONS LTD. BAFFINS LANE CHICHESTER, W SUSSEX PO19 1UD, ENGLAND. ISSN: 1088-4246. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND TALL FORMATS

AB The texaphyrins are prototypical metal-coordinating expanded porphyrins. They represent a burgeoning class of pharmacological agents that show promise for an array of medical applications. Currently, two different water-soluble lanthanide texaphyrins, namely motexafin gadolinium (Gd-Tex, 1) and motexafin lutetium (Lu-Tex, 2), are involved in multi-center clinical trials for a variety of indications. The first of these agents, XCYTRIN R) motexafin gadolinium Injection, is being evaluated as a potential X-ray radiation enhancer in a randomized Phase III clinical trial in patients with brain metastases. The second, in various formulations, is being evaluated as a **photosensitizer** for use in: (i) the photodynamic **treatment** of recurrent breast cancer (LUTRIN R) Injection; now in Phase IB clinical trials); (ii) photoangioplastic reduction of atherosclerosis involving peripheral and coronary arteries (ANTRIN R) Injection; now in Phase II and Phase I clinical trials, respectively); and (iii) light-based **age-related macular degeneration** (OPTRIN R) Injection; currently under Phase II clinical evaluation, a vision-threatening disease of the retina. In this article, these developments, along with fundamental aspects of the underlying chemistry are reviewed. Copyright (C) 2001 John Wiley & Sons, Ltd.

L9 ANSWER 17 OF 44 SCIFINDER COPYRIGHT 2002 ACS (R)
2001:456611 The Genuine Article (R) Number: 447AN. **Photosensitizer** delivery for photodynamic therapy of choroidal neovascularization. Kenno K D; Miller J W (Reprints). Harvard Univ, Massachusetts Eye & Ear Infirm, Sch Med, Angiogenesis Lab, Retina Serv, Boston, MA 02114 USA (Reprints). ADVANCED DRUG DELIVERY REVIEWS (1 OCT 20 01) Vol. 52, No. 1, pp. 63-78. Publisher: ELSEVIER SCIENCE BV. PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. ISSN: 0169-409X. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND TALL FORMATS

AB The present review examines the importance of improving **photosensitizer** delivery for choroidal neovascularization (CNV) in light of the clinical impact of photodynamic therapy (PDT) for CNV. An overview of the classes of available **photosensitizers** is provided and the properties governing **photosensitizer** uptake and circulation in serum are discussed. Current delivery systems, for example

liposomal formulations as well as the use of the promising strategy of antibody targeted delivery as a strategy to improve PDT selectivity and efficiency for CNV **treatment** are described. A summary of the work using Verteporfin, tin ethyl purpurin and Lu-Tex - **photosensitizers** currently in clinical trials for CNV is given.

L9 ANSWER 28 OF 58 MEDLINE DUPLICATE 1
2001076926 Document Number: 20576064. PubMed ID: 11133846. A new drug-screening procedure for photosensitizing agents used in photodynamic therapy for CNV. Lange N; Ballini J P; Wagnieres G; van den Bergh H. (Institute of Environmental Engineering, Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland.. norbert.lange@epfl.ch). INVESTIGATIVE OPHTHALMOLOGY AND VISUAL SCIENCE, 2001 Jan 42 (1) 38-46. Journal code: 7703701. ISSN: 0146-0404. Pub. country: United States. Language: English.

AB PURPOSE: Because vascular occlusion has been observed as a consequence of photodynamic therapy (PDT), this method has been successfully used for the **treatment** of choroidal neovascularization (CNV) in **age-related macular degeneration** (AMD). However, most conventional **photosensitizers**, primarily developed for tumor PDT, lack selectivity for the targeting of neovascularization. An experimental model has been developed for drug screening of new **photosensitizers** for the **treatment** of CNV associated with AMD. It consists of intravenous (IV) injection of **photosensitizers** and fluorescent dyes into the chick's chorioallantoic membrane (CAM), followed by measurement of fluorescence pharmacokinetics, leakage from the vascular system, and photothrombic efficacy. METHODS: Fertilized chicken eggs were placed under a fluorescence microscope. After intravenous injection of different dyes, time-dependent fluorescence angiography was performed. The effect of PDT parameters was assessed by fluorescence angiography 24 hours after PDT. RESULTS: Although fluorescence of lipophilic benzoporphyrin derivative monoacid ring A (BPD-MA) remained intravascular during 2 hours, hydrophilic dyes tended to leak through the fenestrated neovascularization. By variation of PDT parameters, vascular damage could be directed toward closure of vessels with a diameter smaller than 10 microm, as measured 24 hours after PDT. High **photosensitizer** concentrations and high light doses resulted in blood flow stasis within 60 minutes, performed by fluorescence angiography. CONCLUSIONS: Fluorescence angiography and PDT after IV injection into the CAM showed strong similarities to results obtained in clinical tests of PDT in CNV associated with AMD. Thus, this model can provide valuable information about PDT mechanisms and can be used for drug-screening purposes in development of improved sensitizers for the PDT of CNV.

L9 ANSWER 29 OF 58 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2001:14499 Document No.: PREV200100046499. Photodynamic therapy with verteporfin for **age-related macular degeneration**. American Academy of Ophthalmology. Ophthalmology, December, 2000 Vol. 107, No. 12, pp. 2314-2317. print. ISSN: 0161-6420. Language: English. Summary Language: English.

AB Objective: This document describes photodynamic therapy (PDT) with verteporfin for **age-related macular degeneration** (AMD) and examines the evidence to answer the key question about whether the **treatment** is safe and effective in reducing visual loss from AMD. Methods: A literature search that was conducted in April 2000 retrieved eight relevant citations, and the reference lists of these articles were consulted for additional citations. Panel members reviewed this information, and a methodologist reviewed and rated all articles according to the strength of evidence. Results: The published literature contains a report of the combined results from two identically designed, double-masked randomized controlled trials.

Ninety-four percent of participants completed the one-year follow-up. Patients treated with verteporfin had a decreased risk of at least moderate visual loss over this one-year period, but the beneficial effect on visual acuity was greatest among eyes in which the area of classic choroidal neovascularization (CNV) occupied 50% or more of the entire lesion area. There was no statistically significant difference in visual acuity outcomes at one year for eyes in which the classic CNV was more than 0% but less than 50% of the area of the entire lesion. Serious systemic complications were rare. Severe vision decrease (equivalent to four lines or more of vision) within 7 days of **treatment** with verteporfin has been reported in 1% to 4% of patients. Partial recovery of vision was observed in many of these patients. Conclusions: To date, evidence suggests that PDT using verteporfin can reduce the risk of visual loss in patients with predominantly classic subfoveal CNV from AMD at one year. The rate of ocular and systemic complications is low. Additional clinical research is needed to determine the long-term effectiveness of **treatment** and the comparative effectiveness with existing and new **treatment** modalities under investigation.

L9 ANSWER 30 OF 66 BIOSIS COPYRIGHT 2012 BIOLOGICAL ABSTRACTS INC.
2001:117161 Document No.: PREV200100157161. New examination methods for macular disorders: Application of diagnosis and **treatment**.
Yoshida, Akitoshi (1). (1) Department of Ophthalmology, Asahikawa Medical College, 2-1 Midorigaoka Higashi, Asahikawa, 078-8510 Japan. Nippon Ganka Gakkai Zasshi, December, 2000) Vol. 104, No. 12, pp. 899-941. print. ISSN: 0029-0203. Language: Japanese. Summary Language: English; Japanese.

AB To establish a diagnosis or evaluate the efficacy of **treatment** for macular disorders, we need methods to evaluate the anatomical and functional changes of these disorders. In this article, we describe several studies that we have conducted for 2 years. In section 2, we report our new methods for making a diagnosis and evaluating visual function in macular disorders. In section 3, we describe our trials of these examination methods in **treatment**. Here is the summary of our results. In section 1, to examine the structures of the macular area, we used a retinal thickness analyzer (RTA), a confocal scanning laser ophthalmoscope Heidelberg Retina Tomograph, HRT), and optical coherence tomography (OCT) to measure retinal thickness and assess retinal microstructures. We compared retinal imaging analysis of various macular diseases obtained with these three instruments. With the RTA, we obtained good three-dimensional macular images displayed on a retinal thickness map, but the retinal thickness map did not demonstrate the thickened retina with dense retinal hemorrhages, and high backscattering from hard exudates might obscure the vitreoretinal interface. The HRT three-dimensional topographic image clearly shows the undulation of the retinal surface. However, it took a relatively long time to obtain the HRT image, and we sometimes could not obtain good topographic images because of fixation movement. Examination with the OCT allows confirmation of the retinal cross-sectional structures, such as retinoschisis or cystoid spaces and the vitreomacular interface, such as vitreous traction, that cannot be detected using other conventional methods with high resolution, but high reflectivity from dense hemorrhages obscured the deeper layers of the retinal structures. Measurement of retinal thickness obtained with both the RTA and OCT is highly reproducible, and there was significant correlation between the retinal thicknesses measured with the two instruments. We believe that these three instruments might contribute significantly to early, accurate diagnosis and better monitoring of the therapeutic effects of vitrectomy for macular diseases. In the future, if these fundus imaging analysis instruments can achieve higher resolution and can analyze three-dimensional retinal images, they will provide better information to clinically evaluate macular diseases. We demonstrated vitreous examination and examination from the retinal surface to the deeper retinal layer at the macular area using a scanning laser ophthalmoscope (SLO). The SLO examination with an argon laser and a large

confocal aperture was useful for conducting kinetic examination of the vitreous opacity above the macula. With a diode laser and a ring aperture (dark-field mode), it was possible to examine the retina from the deeper retinal layer to the choroids. On the other hand, the SLO also allows us to conduct a functional examination of fixation. We demonstrated that the preferred retinal locus of fixation may change during the follow-up period in patients whose central fixation is impaired due to macular disease, and we showed that the fixation behavior was related to the visual acuity. Therefore, the SLO is an ideal instrument for determining the visual field and the visual acuity before and after **treatment** in patients with macular disease, because of its precise localization of the examination point by directly observing the fundus and by monitoring fixation behavior. Our new program installed in the SLO allows us to complete the quantitative retinal sensitivity evaluation within 2 minutes, which is difficult to do using a conventional SLO program. Furthermore, we demonstrated for the first time that minute functional changes in the retina can be detected by the SLO under low background illuminance. Such changes cannot be detected under conventional conditions. In addition, the extrafoveal visual acuity of normal subjects and patients with macular disease was studied using this new SLO program. The isoacuity lines could be illustrated by summarizing these results in normal subjects. The SLO acuity of the horizontal meridian is significantly better than that of the vertical meridian, and even in the nasal area adjacent to the optic disc, an acuity of better than .1 could be achieved. To evaluate macular function, we also investigated the blood flow of the choroid (CF), the retina (RF), and the choriocapillaris at the fovea (CCF). We investigated the CF in patients with **age-related macular degeneration** (AMD) using pulsatile ocular blood flow (POBF) measurements. In patients with exudative AMD, the POBF was significantly lower than in patients with nonexudative AMD or in control subjects. Decreased CF may play a role in the development of choroidal neovascularization in AMD. RF was measured using laser Doppler velocimetry (LDV). We found that the RF in diabetes changes depending on the stage of diabetic retinopathy, the duration of diabetes, and the **treatment** of retinopathy. We developed a new LDV instrument equipped with an eye-tracking system, and demonstrated good reproducibility with this instrument. CCF was measured using the newly developed laser Doppler flowmetry (LDF), which also had good reproducibility. We measured CCF in patients with AMD in one eye, and found that the CCF in the eyes with AMD is sometimes lower than the CCF in normal eyes. We also measured CCF in patients with macular edema (ME) based on branch retinal vein occlusion in one eye, and found that CCF in these eyes was significantly lower than CCF in normal eyes. To evaluate the dysfunction of the blood retinal barrier (BRB) in diabetic ME, we developed a new differential vitreous fluorophotometry that can simultaneously measure fluorescein and fluorescein monoglucuronide in the vitreous. We investigated the inward and outward permeability of the BRB in patients with diabetic ME. In patients with diabetic ME, the dysfunction of both the inward and the outward permeability of the BRB was demonstrated using differential vitreous fluorophotometry. In section 2, we first presented the potential of the newly developed macular photocoagulation technique. We showed that it is possible to apply macular photocoagulation more safely using the SLO even in patients with unstable fixation, when it is performed in combination with the new three-dimensional eye-tracking system. We then presented the results of photodynamic therapy (PDT) used to treat choroidal neovascularization (CNV) in an animal model using a new **photosensitizer** developed by us. Finally, we demonstrated the newly developed vitreous surgery simulation system using virtual-reality technology. The simulator can provide ophthalmologists with a new surgical training method for preretinal membrane peeling and CNV removal. From these studies, we showed the value of the new instruments for examining patients with macular disorders, pointed out problems that face our clinicians, and proposed new goals for the future. Establishment of these

new examinations can provide the basis for the development of new **treatments**. Advances in medical technology will enable diagnosis and **treatment** of macular disorders to be more progressive.

L9 ANSWER 51 OF 58 MEDLINE DUPLICATE 9
20001251259 Document Number: 20181259. PubMed ID: 10718131. Texaphyrins: new drugs with diverse clinical applications in radiation and photodynamic therapy. Sessler J L; Miller R A. Department of Chemistry & Biochemistry, University of Texas, Austin 78712, USA.. sessler@mail.utexas.edu . BIOCHEMICAL PHARMACOLOGY, (2000 Apr 1) 59 (7) 733-9. Ref: 41. Journal code: 1101 3. ISSN: 0006-2952. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The texaphyrins are quintessential metal-coordinating expanded porphyrins. They constitute a new series of synthetic porphyrin analogues that show promise as drugs for use in a range of medical therapies. Currently, two different water-solubilized lanthanide(III) texaphyrin complexes, namely the gadolinium III and lutetium(III) derivatives 1 and 2 (Gd-Tex and Lu-Tex, respectively), are being tested clinically. The first of these, XCYTRIN, is in a pivotal Phase III clinical trial as a potential enhancer of radiation therapy for patients with metastatic cancers to the brain receiving whole brain radiation therapy. The second, in various formulations, is being tested as a **photosensitizer** for use in: (1) the photodynamic **treatment** of recurrent breast cancer (ANTRIN; Phase II clinical trials complete), (2) photoangioplastic restriction of atherosclerosis involving peripheral arteries (ANTRIN; now in Phase II testing), and (3) light-based **treatment** of **age related macular degeneration** (OPTRIN; currently in Phase I clinical trials), a vision-threatening disease of the retina. Taken in concert, these two metallotexaphyrins provide a powerful new class of experimental drugs whose diverse potential utility is abetted by a combination of well optimized physical features, favorable tissue biodistribution characteristics, and novel mechanisms of action. Interestingly, these mechanisms may alter conventional wisdom regarding mechanisms of radiation therapy and the pathophysiology of atherosclerosis.

L9 ANSWER 52 OF 58 MEDLINE DUPLICATE 10
20002274211 Document Number: 20227421. PubMed ID: 10843515. Selective photodynamic effects of the new **photosensitizer** ATX-S10 Na(I) on experimental neovascularization in monkeys. Obana A; Gohto Y; Kohmi M; Nakajima S; Haseda H; Miki T. Department of Ophthalmology, Osaka City University Medical School, Japan.. akira-kun@med.osaka-cu.ac.jp . ARCHIVES OF OPHTHALMOLOGY, (2000 May) 118 (5) 650-8. Journal code: 17;6154. ISSN: 0003-9950. Pub. country: United States. Language: English.

AB OBJECTIVE: To determine the optimal **treatment** variables for photodynamic therapy (PDT) with new **photosensitizer** ATX-S10(Na) [3,17-bis(1-carboxypropionyl) carbamoylethyl-8-ethenyl-3-hydroxy-2-hydroxyiminoethylidene-2,7,12,18-tetramethyl-6 porphyrin sodium] to induce selective occlusion of choroidal neovascularization (CNV) in nonhuman primate eyes. METHODS: Experimental CNV was induced in monkey eyes by laser photocoagulation, and PDT was performed in neovascularized and healthy eyes with different **treatment** variables. At 0 to 150 minutes after 4-, 8-, and 12-mg/kg of body weight intravenous injections of ATX-S10 Na(I), a diode laser was irradiated at the dose of 1 to 127 J/cm2 (wavelength, 670 nm). Vascular occlusion induced by PDT was evaluated using fluorescein angiography, indocyanine green angiography, and histological examination at 1 day to 4 weeks after irradiation. RESULTS: Selective occlusion of CNV without damage to healthy retinal and choroidal capillaries was achieved in the following conditions: 30 to 74 J/cm2 irradiation at 30 to 74 minutes after the 8-mg/kg injection, and 1 to 29 J/cm2 irradiation at 30 to 74 minutes or 30 to 74 J/cm2 irradiation at 75 to 150 minutes after the 12-mg/kg dye injection. Regrowth of CNV often occurred when the retina was heavily injured by excessive PDT. CONCLUSION:

By using optimal **treatment** variables, PDT using ATX-S10(Na) induces selective occlusion of CNV in nonhuman primate eyes, providing the possibility of therapeutic application to the clinical practice. CLINICAL RELEVANCE: Occlusion of CNV without direct damage to the sensory retina is useful to preserve visual acuity in patients with exudative **age-related macular degeneration**. A clinical trial of PDT using ATX-S10(Na) is desirable.

L9 ANSWER 55 OF 58 BIOSIS COPYRIGHT 2001 BIOLOGICAL ABSTRACTS INC.
2000:245519 Document No.: PREV200000245519. Photodynamic therapy of subfoveal choroidal neovascularization in **age-related macular degeneration** using verteporfin (Visudyne): Two-year results of 2 randomized clinical trials: TAP report 3. Blumenkranz, M. S. (1); Bressler, S. B. (2); TAP Study Group (1) (1) Wilmer Eye Institute-Johns Hopkins University School of Medicine, Baltimore, MD USA. IOVS, (March 15, 2000) Vol. 41, No. 4, pp. S568. Meeting Info.: Annual Meeting of the Association in Vision and Ophthalmology. Fort Lauderdale, Florida, USA April 30-May 05, 2000 Association for Research in Vision and Ophthalmology. Language: English. Summary Language: English.

L9 ANSWER 54 OF 58 BIOSIS COPYRIGHT 2001 BIOLOGICAL ABSTRACTS INC.
2000:245518 Document No.: PREV200000245518. Photodynamic therapy of subfoveal choroidal neovascularization in **age related macular degeneration** using verteporfin (Visudyne): Impact of lesion component on one-year visual outcomes: TAP report 2. Lewis, H. (1); TAP Study Group (1) (1) Dole Eye Institute-Cleveland Clinic Foundation, Cleveland, OH USA. IOVS, (March 15, 2000) Vol. 41, No. 4, pp. S567. Meeting Info.: Annual Meeting of the Association in Vision and Ophthalmology. Fort Lauderdale, Florida, USA April 30-May 05, 2000 Association for Research in Vision and Ophthalmology. Language: English. Summary Language: English.

L9 ANSWER 53 OF 58 BIOSIS COPYRIGHT 2001 BIOLOGICAL ABSTRACTS INC.
2000:245516 Document No.: PREV200000245516. Photodynamic therapy with tin ethyl etiopurpurin (SnET2) of subfoveal choroidal neovascularization (CNV) in **age-related** maculopathy: Study design and baseline characteristics. Thomas, E. L. (1); Murphy, P. P.; Bressler, S. B.; Eriksson, M.; Rauch, A. M. (1) Retina Vitreous Associates, Beverly Hills, CA USA. IOVS, (March 15, 2000) Vol. 41, No. 4, pp. S563. Meeting Info.: Annual Meeting of the Association in Vision and Ophthalmology. Fort Lauderdale, Florida, USA April 30-May 05, 2000 Association for Research in Vision and Ophthalmology. Language: English. Summary Language: English.

L9 ANSWER 52 OF 58 BIOSIS COPYRIGHT 2001 BIOLOGICAL ABSTRACTS INC.
2000:245477 Document No.: PREV200000245477. Photodynamic therapy of subfoveal choroidal neovascularization in **age related macular degeneration** using verteporfin (Visudyne): Exploratory analysis of good visual outcomes: TAP report 4. Singerman, L. (1); TAP Study Group (1) (1) Retina Associates of Cleveland, Cleveland, OH USA. IOVS, (March 15, 2000) Vol. 41, No. 4, pp. S561. Meeting Info.: Annual Meeting of the Association in Vision and Ophthalmology. Fort Lauderdale, Florida, USA April 30-May 05, 2000 Association for Research in Vision and Ophthalmology. Language: English. Summary Language: English.

L9 ANSWER 47 OF 58 CAPLUS COPYRIGHT 2002 ACS
2001:134765 Document No. 134:143907 Photodynamic therapy with verteporfin for choroidal neovascularization caused by **age-related macular degeneration**: results of a single **treatment** in a phase 1 and 2 study. [Erratum to document cited in CA131:234694]. Miller, Joan W.; Schmidt-Erfurth, Ursula; Sickenberg, Michel; Pournaras, Constantin J.; Laqua, Horst; Barbazetto, Irene; Zografos, Lecnidas; Piguet, Bertrand; Donati, Guy; Lane, Anne-Marie; Birngruber, Reginald; Van den Berg, Hubert; Strong, H. Andrew; Manjuris,

Ulrike; Gray, Todd; Fsadni, Mario; Bressler, Neil M.; Gragoudsa, Evangelos S. (Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA, USA). Archives of Ophthalmology (Chicago), 118(4), 4 pp. English) 2000. CODEN: ARCHAW. ISSN: 0003-9950. Publisher: American Medical Association.

AB Journal omission of financial disclosure, properly reported at the time of manuscript submission, occurred in the acknowledgment section on page 552. The following statement should have appeared in the article: "Drs. Rosenberg and Bressler are consultants for CIBA Vision, Inc., Duluth, Ga, and QLT Phototherapeutics Inc., Vancouver, British Columbia.".

L9 ANSWER 59 OF 59 SCISEARCH COPYRIGHT 2002 ISI (R)

2000:17650 The Genuine Article (R) Number: 311MD. Recent advances in photodynamic therapy. Bandey H K (Reprint). NEW YORK STATE DEPT HLTH, ROSWELL PK CANC INST, PHOTODYNAM THERAPY CTR, BUFFALO, NY 14263 (Reprint). JOURNAL OF PORPHYRINS AND PHTHALOCYANINES (JUN-JUL 2000) Vol. 4, No. 4, pp. 368-373. Publisher: JOHN WILEY & SONS LTD. BAFFINS LANE CHICHESTER, W SUSSEX PO19 1UD, ENGLAND. ISSN: 1088-4246. Pub. country: USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ASC AND XALL FORMATS

AB Clinical results of photodynamic therapy continue to show promise for the **treatment** of various solid malignancies. This paper briefly summarizes the advantages/disadvantages of various so-called 'second-generation' **photosensitizers** and other medical applications of porphyrin-based analogs. Copyright (C) 2000 John Wiley & Sons, Ltd.

L9 ANSWER 59 OF 59 CAPLUS COPYRIGHT 2002 ACS

2000:12791 Document No. 132:23871 A preliminary study of photodynamic therapy using verteporfin for choroidal neovascularization in pathologic myopia, ocular histoplasmosis syndrome, angioid streaks, and idiopathic causes. Rosenberg, Michael; Schmidt-Erfurth, Ursula; Miller, Joan W.; Pournaras, Jonathan J.; Zografos, Leonidas; Piguet, Bertrand; Donati, Guy; Lapalu, Horst; Barbazetto, Irene; Gragoudas, Evangelos S.; Lane, Anne-Marie; Birngruber, Reginald; Van den Berghe, Hubert; Strong, H. Andrew; Manfurna, Ulrike; Gray, Todd; Fsadni, Mario; Bressler, Neil M. Hopital Ophtalmique Jules Gonin, Lausanne, Switz. . Archives of Ophthalmology (Chicago), 118(5), 636-643 (English) 2000. CODEN: ARCHAW. ISSN: 0003-9950. Publisher: American Medical Association.

AB Objective: To evaluate short-term safety and the effects on visual acuity and fluorescein angiog. of single or multiple sessions of photodynamic therapy with verteporfin for choroidal neovascularization (CNV) not related to **age related macular degeneration** (AMD), including pathol. myopia, the ocular histoplasmosis syndrome, angioid streaks, and idiopathic causes. Design: A nonrandomized, multicenter, open-label, dose escalation phase I and 2 clin. trial. Setting: Four ophthalmic centers in Europe and North America providing retinal care. Participants: Thirteen patients with subfoveal CNV due to pathol. myopia, the ocular histoplasmosis syndrome, angioid streaks, or idiopathic causes. Methods: Standardized protocol refraction, visual acuity testing, ophthalmic exams., color photographs, and fluorescein angiograms were used to evaluate the results of photodynamic therapy **treatments** with verteporfin. Follow-up ranged from 1 wk for patients who were treated once to 43 wk for patients who were treated up to 4 times. Results: Verteporfin therapy was well tolerated in patients with CNV not related to AMD. No deterioration in visual acuity was obsd.; most patients gained at least 1 line of vision. Reduction in the size of leakage area from classic CNV was noted in all patients as early as 1 wk after verteporfin therapy, with complete absence of leakage from classic CNV in almost half of the patients. Improvement in visual acuity after verteporfin therapy was greatest (+6, +6, and +9 lines) in 3 patients with relatively poor initial visual acuity (between 20/200 and 20/800). Up to 4 **treatments** were found to have short-term

safety even with retreatment intervals as short as 4 wk. Conclusions: **Treatment** of CNV not related to AMD with verteporfin therapy achieves short-term cessation of fluorescein leakage from CNV in a small no. of patients without loss of vision. Further randomized clin. trials including a larger no. of patients are under way to confirm whether verteporfin therapy is beneficial for subfoveal CNV not related to AMD.

L9 ANSWER 40 OF 50 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 11
2000142[illegible] EMBASE Porphyrin-mediated photosensitization - Taking the apoptosis fast lane. Granville D.J.; Hunt D.W.C.. D.J. Granville, QLT PhotoTherapeutics Inc., 887 Great Northern Way, Vancouver, BC V5T 4T5, Canada. dgranville@qltinc.com. Current Opinion in Drug Discovery and Development 3:2 (231-242) 2000.
Refs: 138.
ISSN: 1367-6733. CODEN: CODDFF. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB Photodynamic therapy (PDT), which is an approved anticancer **treatment**, is also an effective approach to treat certain immune-mediated (psoriasis), ocular **age-related macular degeneration** and cardiovascular (removal of atherosclerotic plaque and prevention of restenosis following angioplasty) conditions. PDT uses light-absorbing **photosensitizers**, often a porphyrin derivative, which accumulate somewhat selectively within proliferating cell types. Upon illumination with light of an activating wavelength, reactive oxygen species are produced in **photosensitizer**-containing cells. Cell death may ensue. PDT with various **photosensitizers** causes cells to die rapidly by apoptosis, a built-in suicide program during which the cell disassembles itself. This review considers the notable properties of **photosensitizers** that relate to their potent capacity to induce cell death upon photoactivation. **Photosensitizers** can trigger apoptosis by a direct action upon mitochondria, a feature enabling PDT to be an effective **treatment** for disease conditions in which anti-apoptotic mechanisms to standard chemotherapeutic agents are present. The contribution of cell signaling events to the photodynamic effect and the relationship of PDT to other apoptosis pathways are also considered. Unraveling the biochemistry of PDT-induced apoptosis fosters the identification of disease indications, as well as promoting the potential for the application of PDT in combination with other therapeutic agents.

L9 ANSWER 41 OF 50 MEDLINE DUPLICATE 12
20011[illegible] Document Number: 20947147. PubMed ID: 11094144. Mechanisms of action of photodynamic therapy with verteporfin for the **treatment** of **age-related macular degeneration**
. Schmidt-Erfurth U; Hasan T. (University Eye Hospital, Lubeck, Germany.) SURVEY OF OPHTHALMOLOGY, (2000 Nov-Dec) 45 (3) 195-214. Ref: 97. Journal code: [illegible]4551. ISSN: 0039-6257. Pub. country: United States. Language: English.

AB **Age-related macular degeneration**, especially the neovascular form of the disease, is the leading cause of blindness in elderly people in developed countries. Thermal photocoagulation is still the preferred **treatment** for choroidal neovascularization that does not involve the fovea, but it is suitable for only a small number of patients and it can lead to immediate loss of visual acuity. Photodynamic therapy with use of photochemical light activation of verteporfin as a **photosensitizer** (verteporfin therapy) has been shown to be effective in treating vascularized tumors, and its potential to treat other conditions involving neovascularization has also been suggested. Preclinical and clinical studies have indicated that verteporfin therapy can be used to treat choroidal neovascularization secondary to **age-related macular degeneration** effectively and safely. Selective occlusion of choroidal neovasculature by this therapy causes minimal damage to the

neurosensory retina and, therefore, does not induce loss of visual acuity. This benefit allows verteporfin therapy to be used in the large proportion of patients who are not eligible for **treatment** by laser photocoagulation. The mechanistic aspects of the mode of action of light-activated verteporfin are described in this review.

L9 ANSWER 42 OF 58 CAPLUS COPYRIGHT 2011 ACS
2000:304917 Document No. 132:86117 Verteporfin. Scott, Lesley J.; Goa, Karen L. Adis International Limited, Auckland, N. Z.). Drugs & Aging, 16(2), 139-146 (English). 2000. CODEN: DAGEE6. ISSN: 1170-229X. Publisher: Adis International Ltd..

AB A review with 22 refs. Verteporfin, a benzoporphyrin deriv. monoacid ring A, is a photosensitizing drug for photodynamic therapy (PDT) activated by low-intensity, nonheat-generating light of 689nm wavelength. Activation generates cytotoxic oxygen free radicals. The specificity and uptake of verteporfin for target cells with a high expression of low d. lipoprotein (LDL) receptors, such as tumor and neovascular endothelial cells, is enhanced by the use of a liposomal formulation and its rapid uptake by plasma LDL. Verteporfin therapy at light doses of 600 J/cm2 selectively damages neovascular endothelial cells leading to thrombus formation and specific occlusion of choroidal neovascular vessels in subfoveal lesions in patients with **age related macular degeneration** (AMD). Repeated applications of verteporfin therapy (6 mg/m2) improved or maintained visual acuity in the majority of patients with some classic subfoveal choroidal neovascularization (CNV) secondary to AMD at 1 yr's follow-up in 2 large multicenter, placebo-controlled, double-blind trials. Furthermore, in a subgroup of these patients with predominantly classic CNV secondary to AMD, there was a significantly more marked visual acuity (VA) benefit with 6/7.5 of verteporfin-treated eyes experiencing less than a 15-letter loss of VA vs. 2/9 with placebo **treatment**. Multiple applications of verteporfin therapy were well tolerated in patients with subfoveal CNV secondary to AMD. The most common adverse events were visual disturbances, injection site reactions, photosensitivity reactions and infusion related back pain.

L9 ANSWER 43 OF 58 MEDLINE DUPLICATE 13
2004:338677 Document Number: 20477274. PubMed ID: 15221665. [Choroidal changes after photodynamic therapy (PDT). A two-year following study of 38 patients]. Aderhaltveranderungen nach photodynamischer Therapie (PDT). Verlaufsbeobachtungen uber 2 Jahre bei 38 Patienten. Michels S; Barbazetto I; Schmidt-Erfurth U. (Klinik fur Augenheilkunde, Medizinische Universitat zu Lubeck.) KLINISCHE MONATSBLATTER FUR AUGENHEILKUNDE, 2004 Aug) 221 (8) 574-9. Journal code: 0114154. ISSN: 0023-2165. Pub. Country: GERMANY: Germany, Federal Republic of. Language: German.

AB BACKGROUND: Photodynamic therapy is a new option for **treatment** of choroidal neovascularisation in patients with **age related macular degeneration**. But choroidal changes and associated angiographic characteristics have not been further evaluated. PATIENTS: Indocyanine green angiography was used to follow 38 patients with subfoveal choroidal neovascularisation in **age related macular degeneration** over up to two years. All patients were treated with the **photosensitizer** Benzoporphyrin Derivative-MA receiving either a single or triple **treatment**. RESULTS: Indocyanine green angiography shows two effects of photodynamic therapy. On the one hand a selective and lasting closure of choroidal neovascularisation was documented. Choroidal neovascularisation-size and leakage was significantly reduced in the entire **treatment** group to 46.7% and 23.8% one week after **treatment**, followed by a slow increase to 83.3% and 41.2% at up to two years longterm follow up. On the other hand photodynamic therapy causes typically a peri-lesional hypofluorescence in Indocyanine green angiography. This hypofluorescence is most likely due to choroidal hypoperfusion and vascular endothelial changes. A continuous increase in

fluorescence was shown, reaching again 90% of the pretreatment intensity at 3 months, documenting a good recovery of the choroidal network. CONCLUSION: The results show that photodynamic therapy is an alternative **treatment** in **age-related macular degeneration** with choroidal, subfoveal neovascularisation. Indocyaninegreen angiography reflects well choroidal changes associated with this therapy and may be helpful to choose **treatment** intervals.

L9 ANSWER 44 OF 52 CABLUS COPYRIGHT 2002 ACS
1999:761,38 Document No. 132:44149 Changing therapeutic paradigms for exudative **age-related macular degeneration**: antiangiogenic agents and photodynamic therapy. Challa, Thomas A.; Danis, Ronald P.; Criswell, Mark; Pratt, Linda M. (Indiana University Macular Degeneration Clinic and Research Center and The Vitreo-Retinal Service, Department of Ophthalmology, Indiana University School of Medicine, Indianapolis, IN, USA). Expert Opinion on Investigational Drugs, 8(12), 2179-2192 (English) 1999. CODEN: EOIDER. ISSN: 1354-3784. Publisher: Ashley Publications.

AB A review with 43 refs. **Age related macular degeneration** AMD) is the leading cause of irreversible visual loss in the United States. Overall, approx. 10 - 20% of patients with AMD exhibit the exudative form, which is responsible for most of the estd. 1.2 m cases of severe visual loss from AMD. Visual loss develops in the exudative form of AMD due to abnormal choroidal neovascular membranes (CNM) that develop under the retina, leak serous fluid and blood, and ultimately cause a blinding disciform scar in, and under, the retina. Currently, the only well-studied and widely accepted method of **treatment** is laser photocoagulation of the CNM. However, only a minority of patients with exudative AMD show well-demarcated "classic" CNM amenable to laser **treatment**, and at least half of these patients suffer persistent or recurrent CNM formation within two years. In addn., since the **treatment** itself causes a blinding central scotoma when the CNM is located subfoveally, many clinicians do not treat subfoveal CNM. With these **treatment** limitations, there has been a great deal of interest in alternative therapies for AMD, including anti-angiogenic agents and photodynamic therapy. Angiogenesis involves a complex interplay of cellular events involving a cascade of factors that are both inhibitory and stimulatory. Fibroblast growth factors have been the best-known cell mediating agents in ophthalmol., but there are a multitude of potential sites for inhibition of angiogenesis by pharmacol. agents. With regard to photodynamic therapy, a photosensitizing dye is injected intravascularly and low power laser light is used to activate the dye within the CNM to cause vascular occlusion by a photochem. reaction. Closure of the CNM is achieved without severe collateral damage to the neurovascular tissues as occurs with laser photocoagulation.

L9 ANSWER 45 OF 52 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 14
2000:145916 The Genuine Article (R) Number: 514RH. Expanded porphyrins. Synthetic materials with potential medical utility. Sessler J L (Reprint); Tvermoes N A; Davis J; Anzenbacher P; Furuhkova K; Sato W; Veauthier D; Lynch V; Black C B; Try A; Andrioletti B; Hemmi G; Mody T D; Miller D J; Kral V. UNIV TEXAS, DEPT CHEM & BIOCHEM, AUSTIN, TX 78712 (Reprint); UNIV TEXAS, INST CELLULAR & MOL BIOL, AUSTIN, TX 78712; PHARMACYCL INC, SUNNYVALE, CA 94089; INST CHEM TECHNOL, DEPT ANALYT CHEM, CR-16628 PRAGUE 6, CZECH REPUBLIC. PURE AND APPLIED CHEMISTRY (NOV 1999) Vol. 71, No. 11, pp. 2009-2014. Publisher: INT UNION PURE APPLIED CHEMISTRY. 104 TW ALEXANDER DR, PO BOX 13757, RES TRIANGLE PK, NC 27709-3757. ISSN: 0033-4545. Pub. country: USA; CZECH REPUBLIC. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A number of aromatic and nonaromatic expanded porphyrins have been prepared in the authors' laboratories. These are allowing a number of

important themes to be explored, including the construction of novel cation- and anion-complexing agents and the generation of drug candidates with considerable therapeutic potential. In this paper, the use of gadolinium(III) and lutetium(III) texaphyrin derivatives as, respectively, adjuvants for X ray radiation cancer therapy and **photosensitizers** for use in photodynamic **treatments** of cancer, atheromatous plaque, and **age-related macular degeneration** will be reviewed. Also discussed are the use of water soluble sapphyrins as potential fluorescent phosphate sensors and organic soluble 2,3-dipyrrylquinoxaline derivatives as possible fluoride anion signaling agents. Recent synthetic work, designed to produce expanded porphyrins with new shapes and novel topologies, is also summarized.

L9 ANSWER 46 OF 58 BIOSIS COPYRIGHT 2001 BIOLOGICAL ABSTRACTS INC.DUPLICATE 15

1999:534617 Document No.: PREV199900534617. Photodynamic therapy of subfoveal choroidal neovascularization in **age-related macular degeneration** with verteporfin: One-year results of 2 randomized clinical trials: TAP report 1. Treatment of Age-related Macular Degeneration With Photodynamic Therapy (TAP) Study Group (1 . (.) Inq.: Neil M. Bressler, 55 N Broadway, Ninth Floor, Baltimore, MD, 21205-2010 USA. Archives of Ophthalmology, Oct., 1999 Vol. 117, No. 10, pp. 1329-1345. ISSN: 0003-9950. Language: English. Summary Language: English.

AB Objective: To determine if photodynamic therapy with verteporfin (Visudyne; QLT A Vision Corp, Duluth, Ga can safely reduce the risk of vision loss in patients with subfoveal choroidal neovascularization (CNV) caused by **age-related macular degeneration** (AMD . Design: Two multicenter, double-masked, placebo-controlled, randomized clinical trials. Setting: Twenty-two ophthalmology practices in Europe and North America. Participants: Patients with subfoveal CNV lesions caused by AMD measuring 5400 mum or less in greatest linear dimension with evidence of classic CNV and best-corrected visual acuity of approximately 20/40 to 20/200. Methods: Six hundred nine patients were randomly assigned 2:1 to verteporfin (6 mg per square meter of body surface area or placebo in dextrose in water administered via intravenous infusion of 10 mL over 10 minutes. Fifteen minutes after the start of the infusion, a laser light at 689 nm delivered 50 J/cm2 at an intensity of 600 mW/cm2 over 83 seconds using a spot size with a diameter 1000 mum larger than the greatest linear dimension of the CNV lesion. At follow-up examinations every 3 months, retreatment with the same regimen was applied if angiography showed fluorescein leakage. The primary outcome was the proportion of eyes with fewer than 15 letters lost approximately 3 lines of loss , adhering to an intent-to-treat analysis. Results: In each group, 88% of patients completed the month 12 examination. Visual acuity, contrast sensitivity, and fluorescein angiographic outcomes were better in the verteporfin-treated eyes than in the placebo-treated eyes at every follow-up examination through the month 12 examination. At the month 12 examination, 246 (61% of 402 eyes assigned to verteporfin compared with 98 (40% of 307 eyes assigned to placebo had lost fewer than 15 letters of visual acuity from baseline (P<0.0 . In subgroup analyses, the visual acuity benefit (<15 letters lost of verteporfin therapy was clearly demonstrated 67% vs 39%; P<0.001 when the area of classic CNV occupied 50% or more of the area of the entire lesion (termed predominantly classic CNV lesions), especially when there was no occult CNV. No statistically significant differences in visual acuity were noted when the area of classic CNV was more than 0% but less than 50% of the area of the entire lesion. Few ocular or other systemic adverse events were associated with verteporfin **treatment**, compared with placebo, including transient visual disturbances (13% vs 12%, injection-site adverse events (13% vs 3%), transient photosensitivity reactions (3% vs 0%), and infusion-related low back pain (2% vs 0%). Conclusions: Since verteporfin

therapy of subfoveal CNV from AMD can safely reduce the risk of vision loss, we recommend verteporfin therapy for **treatment** of patients with predominantly classic CNV from AMD.

L9 ANSWER 4 OF 18 CAPLUS COPYRIGHT 2001 ACS
1999:64144. Document No. 131:54295 Photodynamic therapy with verteporfin for choroidal neovascularization caused by **age-related macular degeneration**: Results of retreatments in a phase I and II study. Schmidt-Erfurth, Ursula; Miller, Joan W.; Sickenberg, Michel; Laqua, Horst; Barbazetto, Irene; Gragoudas, Evangelos S.; Strobos, Katrina; Piguet, Bertrand; Bournaras, Constantin C.; Donati, Guy; Lane, Anne-Marie; Birngruber, Reginald; Van den Berg, Hubert; Strong, N. Andrew; Manjuris, Ulrike; Gray, Todd; Fsadni, Mario; Bressler, Neil M. Retina Department, University Eye Hospital, Lubeck, Germany . Archives of Ophthalmology (Chicago), 117(9), 1177-1187 (English) 1999. CODEN: AROPAW. ISSN: 0003-9950. Publisher: American Medical Association.

AB Objectives: To evaluate safety and short-term visual acuity and fluorescein angiog. effects of photodynamic therapy (PDT) after retreatments with verteporfin for choroidal neovascularization (CNV) in **age-related macular degeneration** (AMD) that demonstrated fluorescein leakage after at least 1 course of PDT. Design: Non-randomized, multicenter, open-label phase I and II clin. trial using 2 different retreatment dosage regimens. Setting: Four ophthalmic centers in Europe and North America providing retinal care. Methods: Standardized protocol refraction, visual acuity testing, ophthalmic exams., color photographs, and fluorescein angiograms were used to evaluate the results of multiple PDT **treatments**. Two regimens (regimens 2 and 4) for **treatment** and retreatment were chosen from 5 used in a single-**treatment** study. Both regimens used a verteporfin dose of 6 mg/m2 infused for 10 min. However, regimen 2 used a light dose of 100 J/cm2 applied 20 min after the start of the verteporfin infusion, whereas regimen 4 used a light dose of 50, 75, or 100 J/cm2 applied 15 min after infusion commenced. Post-**treatment** evaluations were planned in 31 participants up to 3 mo after up to 3 retreatments given at 2- or 4-wk intervals after initial PDT **treatment**. Similar posttreatment evaluations were planned after retreatments in 5 addnl. participants who were re-enrolled some time more than 12 wk after an initial PDT **treatment**. Results: The av. visual acuity change for the 31 participants who had retreatment within 2 to 4 wk after the initial **treatment** and a follow-up exam. 6 to 10 wk after the initial **treatment** was 0.2 lines (range, -4 to 4 lines) in regimen 2 and -1.0 line (range, -5 to 3 lines) in regimen 4. Similar outcomes were noted in the 5 re-enrolled participants. Cessation of fluorescein leakage from classic CNV for at least 2 to 4 wk could be achieved without loss of visual acuity after at least 2 **treatments** in 2 (10.5%) of 19 patients. Similar to single-**treatment** effects, the disappearance of leakage was documented regularly at 1 wk after each retreatment. Fluorescein leakage reappeared by 4 to 12 wk after a retreatment in almost all cases. However, compared with baseline, leakage activity appeared to be reduced after multiple PDT courses. For the 31 patients who had follow-up for 3 mo after the last retreatment and had received retreatment 2 to 4 wk after the initial **treatment**, progression of CNV beyond the area identified before the retreatment was noted in 10 (48%) of the 21 eyes with classic CNV in regimen 2 and 9 (90%) of 10 eyes in regimen 4. The rate and severity of ocular or systemic adverse events were not increased by multiple applications. Conclusions: Multiple applications of PDT with verteporfin achieve repetitive, short-term cessation of fluorescein leakage from CNV secondary to AMD, without loss of visual acuity. This strategy can be used in randomized clin. trials investigating the efficacy of verteporfin in PDT for recurrent fluorescein dye leakage from persistent or recurrent CNV, following an initial or subsequent PDT **treatment**, with maintenance of visual acuity. Retreatments may achieve progressive

cessation of leakage and prevent further growth of CNV and subsequent visual loss.

L9 ANSWER 47 OF 58 CAPLUS COPYRIGHT 2002 ACS
1999:64440 Document No. 131:25434 Photodynamic therapy with verteporfin for choroidal neovascularization caused by **age-related macular degeneration**: results of a single **treatment** in a phase 1 and 2 study. Miller, Joan W.; Schmidt-Erfurth, Ursula; Sickenberg, Michel; Pournaras, Constantin J.; Laqua, Horst; Barbazetto, Irene; Zografos, Leonidas; Piguet, Bertrand; Donati, Guy; Lane, Anne-Marie; Birngruber, Reginald; Van den Berg, Hubert; Strong, N. Andrew; Manjuris, Ulrike; Gray, Todd; Fsadni, Mario; Brassler, Neil M.; Gragoudas, Evangelos S. (Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA, USA). Archives of Ophthalmology (Chicago, 1917-), 117(9), 1161-1173 English 1999. CODEN: AROPAW. ISSN: 0003-9950. Publisher: American Medical Association.

AB Objective: To evaluate the safety and short-term visual and fluorescein angiog. effects of a single photodynamic therapy **treatment** with verteporfin with the use of different dosage regimens in patients with choroidal neovascularization. CNV from **age-related macular degeneration**. Design: Non-randomized, multicenter, open-label, clin. trial using 5 dosage regimens. Setting: Four ophthalmic centers in North America and Europe providing retinal care. Participants: Patients with subfoveal CNV caused by **age-related macular degeneration**. Methods: Standardized protocol refraction, visual acuity testing, ophthalmic exam., color photographs, and fluorescein angiograms were used to evaluate the effects of a single **treatment** of photodynamic therapy with verteporfin. Follow-up was planned through 3 mo in 97 patients and for less than 3 mo in 31 other patients. Results: The mean visual acuity change and range of changes from baseline at the follow-up exam. at week 12 after a single **treatment** with regimens 1 through 5 was -1.1, -3 to +2, -0.2 (-4 to +5), -1.5 (-7 to +2), +0.4 (-8 to +7), and +2.1 (-1 to +9) lines, resp. Only the highest light dose (150 J/cm2) in regimens 2 and 3, which produced angiog. nonperfusion of neurosensory retinal vessels, caused marked vision loss. Some cessation of fluorescein leakage from CNV was achieved without loss of vision when the light dose used was less than 150J/cm2. Systemic adverse events were rare. Cessation of fluorescein leakage from CNV was noted in all regimens by 1 wk after photodynamic therapy. Fluorescein leakage from at least a portion of the CNV reappeared by 4 to 12 wk after **treatment** in almost all cases. Progression of classic CNV beyond the area of CNV identified before **treatment** was noted in 42 (61%) of the 83 eyes with classic CNV followed up for 3 mo after a single **treatment**. Eyes in which the area of any CNV leakage at 12 wk was less than at baseline had a significantly better visual acuity outcome (+0.3 lines) than eyes in which CNV leakage progressed (-0.8 lines). Conclusions: Photodynamic therapy with verteporfin achieved short-term cessation of fluorescein leakage from CNV without loss of vision or growth of classic CNV in some patients with **age-related macular degeneration**. Except for nonperfusion of neurosensory retinal vessels at a light dose of 150J/cm2, no other adverse events were of concern. Randomized clin. trials to investigate whether this new modality can preserve vision in patients with CNV secondary to **age-related macular degeneration** are justified.

L9 ANSWER 49 OF 58 CAPLUS COPYRIGHT 2002 ACS
1999:740973 Document No. 132:13234 Photodynamic immune modulation (PIM). North, John R.; Hunt, David W. C.; Simkin, Guillermo O.; Ratkay, Leslie G.; Chan, Agnes H.; Lui, Harvey H. D.; Levy, Julia G. (LLT PhotoTherapeutics, Inc., Vancouver, BC, Can.). Proceedings of SPIE-The International Society for Optical Engineering, 3863(Biomedical Optics (BMO '99)), 470-474 (English) 1999. CODEN: PSISDG. ISSN: 0277-786X.

Publisher: SPIE-The International Society for Optical Engineering.

AB Photodynamic Therapy (PDT) is accepted for **treatment** of superficial and lumen-occluding tumors in regions accessible to activating light and is now known to be effective in closure of choroidal neovasculature in **Age Related Macular Degeneration.** PDT utilizes light absorbing drugs (**photosensitizers**) that generate the localized formation of reactive oxygen species after light exposure. In a no. of systems, PDT has immunomodulatory effects; Photodynamic Immune Modulation (PIM). Using low intensity photodynamic regimens applied over a large body surface area, progression of murine autoimmune disease could be inhibited. Further, this **treatment** strongly inhibited the immunol.-mediated contact hypersensitivity response to topically applied chem. haptens. Immune modulation appears to result from selective targeting of activated T lymphocytes and resm. in immunostimulation by antigen presenting cells. Psoriasis, an immune-mediated skin condition, exhibits heightened epidermal cell proliferation, epidermal layer thickening and plaque formation at different body sites. In a recent clin. trial, approx. one-third of patients with psoriasis and arthritis symptoms (psoriatic arthritis) displayed a significant clin. improvement in several psoriasis-related parameters after four weekly uncle-keay PIM **treatments** with verteporfin. The safety profile was favorable. The capacity of PIM to ameliorate other human immune disorders including rheumatoid arthritis is under extensive evaluation.

L9 ANSWER 50 OF 54 BIOSIS COPYRIGHT 2000 BIOLOGICAL ABSTRACTS INC.
1999:207694 Document No.: PREV199900207694. Photodynamic therapy (PDT) with verteporfin of subfoveal choroidal neovascularization in **age-related macular degeneration**: Study design and baseline characteristics the VIP randomized clinical trial. Mones, J. (1); VIP Study Group (1 . 1) Institute de Microcirurgia ocular de Barcelona, Barcelona Spain. IOVS, March 15, 1999 Vol. 40, No. 4, pp. S121. Meeting Info.: Annual Meeting of the Association for Research in Vision and Ophthalmology Fort Lauderdale, Florida, USA May 9-14, 1999 Association for Research in Vision and Ophthalmology. Language: English.

L9 ANSWER 51 OF 51 BIOSIS COPYRIGHT 2000 BIOLOGICAL ABSTRACTS INC.
1999:133184 Document No.: PREV199900133184. PDT in the **treatment** of ocular neovasculature. TAP study group; Levy, Julia. Photochemistry and Photobiology, June, 1999 Vol. 69, No. SPEC. ISSUE., pp. 48S. Meeting Info.: Twenty-Seventh Annual Meeting of the American Society for Photobiology Washington, D.C., USA July 10-15, 1999 American Society for Photobiology. ISSN: 0031-8655. Language: English.

L9 ANSWER 52 OF 54 CAPLUS COPYRIGHT 2000 ACS
1998:571199 Document No. 129:168675 Use of green porphyrins to treat neovasculature in the eye. Levy, Julia; Miller, Joan W.; Gragoudas, Evangelos S.; Hasan, Tayyaba; Schmidt-Erfurth, Ursula (The General Hospital Corp., USA; Quadra Logic Technologies, Inc.; Massachusetts Eye & Ear Infirmary). U.S. US 5776943 A 19980707, 19 pp., Cont.-in-part of U.S. Ser. No. 109,173. (English). CODEN: USXXAM. APPLICATION: US 1995-390591 19950217. PRIORITY: US 1994-209478 19940314.

AB Photodynamic therapy of conditions of the eye characterized by unwanted neovasculature, such as **age-related macular degeneration**, is effected using green porphyrins as photoactive agents, preferably as liposomal compns.

L9 ANSWER 53 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R) DUPLICATE 16
1998:196141 The Genuine Article (R) Number: ZB459. Photodynamic therapy in ocular vascular disease (Reprinted from IEEE Journal of Selected Topics in Quantum Electronics, vol 2, 1996). SchmidtErfurth U (Reprint); Birngruber R; Hasan T. UNIV LUBECK, HOSP EYE, D-23538 LUBECK, GERMANY (Reprint); MED LASERZENTRUM LUBECK, D-23562 LUBECK, GERMANY; HARVARD UNIV, MASSACHUSETTS

GEN HOSP, SCH MED, WELLMAN LABS PHOTOMED, BOSTON, MA 02114. LASER PHYSICS (JAN-FEB 1998) Vol. 8, No. 1, pp. 191-193. Publisher: INTERPERIODICA. PO BOX 1691, BIRMINGHAM, AL 35201-1691. ISSN: 1054-660X. Pub. country: GERMANY; USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Photodynamic therapy (PDT) is a novel therapeutical approach which is noninvasive and potentially selective for neoplastic pathologies. Association of **photosensitizers** with low density lipoprotein (LDL) leads to direct targeting of the treated lesions with enhanced efficiency and selectivity. LDL-mediated PDT is particularly useful in the **treatment** of neovascular structures since LDL receptors are abundantly expressed on vascular endothelial cells. To evaluate the potential of selective photodynamic vasoocclusion in ocular neovascular disease, a sequence of experiments was designed: efficiency of the LDL-carrier was tested in vitro, and the system was then transferred to an in vivo model demonstrating a vascularized neoplasm. Occlusion was successfully performed in experimentally-induced neovascularization in the cornea, while selective photothrombosis of subretinal vasculature revealed lack of collateral damage. The experimental results were used to establish a first clinical trial for the use of PDT in **age-related macular degeneration**, one of the leading causes for blindness.

L9 ANSWER 94 OF 95 EMBASE COPYRIGHT (C) 2002 ELSEVIER SCI. B.V.
970991-5 EMBASE Document No.: 1-970995-1. Photodynamic therapy of exudative **age-related macular degeneration**.
Husain D.; Miller J.W., Dr. J.W. Miller, Retina Service, Massachusetts Eye and Ear Infirmary, 243 Charles St, Boston, MA 02114, United States.
Seminars in Ophthalmology 1997 12 (14-16) 1997.
Refs: 17.
ISSN: 0882-053X. CODEN: SEOPET. Pub. Country: United States. Language: English. Summary Language: English.

AB Photodynamic therapy (PDT) is a potentially selective **treatment** modality, which involves systemic administration of a **photosensitizer** dye. Dye accumulates in proliferating tissues such as tumors and neovascularization, followed by exposure of the photosensitized tissue to light at a wavelength at the absorption maximum of the dye. Excitation of the dye leads to photochemical damage of the targeted tissue. Various **photosensitizers** have been used in experimental choroidal neovascularization to investigate PDT. We have used benzoporphyrin derivative monoacid BPD- and shown that it occludes experimental choroidal neovascularization (CNV) with no significant damage to the overlying neurosensory retina or underlying choroid. Clinical trials of PDT using BPD for exudative **age-related macular degeneration** AMD) have started. Preliminary results suggest that CNV can be occluded in the early posttreatment phase, with some nonselective effects at high light doses. Further studies are underway to investigate whether PDT of AMD can help preserve long term vision in patients.

L9 ANSWER 95 OF 95 RESEARCH COPYRIGHT 2002 IST (E) DUPLICATE 17
97:45192 The Genuine Article (R) Number: XD618. Photodynamic therapy in ocular vascular disease. SchmidtErfurth U (Reprint); Birngruber R; Hasan T. UNIV LUBECK, HOSP EYE, D-23538 LUBECK, GERMANY (Reprint); MED LASERZENTRUM LUBECK, D-23562 LUBECK, GERMANY; HARVARD UNIV, MASSACHUSETTS GEN HOSP, SCH MED, WELLMAN LABS PHOTOMED, BOSTON, MA 02114. IEEE JOURNAL OF SELECTED TOPICS IN QUANTUM ELECTRONICS (DEC 1996) Vol. 2, No. 4, pp. 955-966. Publisher: IEEE-INST ELECTRICAL ELECTRONICS ENGINEERS INC, 345 E 47TH ST, NEW YORK, NY 10017-2394. ISSN: 1077-260X. Pub. country: GERMANY; USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Photodynamic therapy (PDT) is a novel therapeutical approach which is noninvasive and potentially selective for neoplastic pathologies,

Association of **photosensitizers** with low density lipoprotein (LDL) leads to direct targeting of the treated lesions with enhanced efficiency and selectivity. LDL-mediated PDT is particularly useful in the **treatment** of neovascular structures since LDL receptors are abundantly expressed on vascular endothelial cells. To evaluate the potential of selective photodynamic vasoocclusion in ocular neovascular disease a sequence of experiments was designed: Efficiency of the LDL-carrier was tested in vitro, the system was then transfered to an in vivo model demonstrating a vascularized neoplasm. Occlusion was successfully performed in experimentally induced neovascularization in the cornea, while selective photothrombosis of subretinal vasculature revealed lack of collateral damage. The experimental results were used to establish a first clinical trial for the use of PDT in **age-related macular degeneration**, one of the leading causes for blindness.

L9 ANSWER 56 OF 57 CAPLUS COPYRIGHT 2002 ACS
1996:427357 Document No. 125:126846 Photodynamic therapy (PDT) as a biological modifier. Oboumi, Modestus; Tao, Ying-Song; Hunt, David; Levy, Julia (QLT Photo Therapeutics, Inc., Vancouver, BC, Can. . Proceedings of SPIE-The International Society for Optical Engineering, 1675(Optical Methods for Tumor Treatment and Detection: Mechanisms and Techniques in Photodynamic Therapy V), 122-131 (English) 1996. CODEN: PSISDG. ISSN: 0277-786X. Publisher: SPIE-The International Society for Optical Engineering.

AB The capacity of **photosensitizers** and light to ablate cancerous tissues and unwanted neovasculature constitutes the classical application of photodynamic therapy (PDT). Cell death results from either necrotic or apoptotic processes. The use of **photosensitizers** and light at doses which do not cause death has been found to affect changes in certain cell populations which profoundly effect their expression of cell surface mols. and secretion of cytokines, thereby altering the functional attributes of the treated cells. Cells of the immune system and the skin may be sensitive to modulation by "sub-lethal PDT". Ongoing studies have been conducted to assess, at the mol. level, changes in both lymphocytes and epidermal cells (EC) caused by **treatment** with low levels of benzoporphyrin deriv. monoacid ring A (BPD) (a **photosensitizer** currently in clin. trials for cancer, psoriasis, endometriosis and **age-related macular degeneration**) and light. **Treatment** of skin with BPD and light, at levels which significantly enhances the length of murine skin allograft acceptance, have been found to down-regulate the expression of Langerhans cell (LC) surface antigen mols. (major histocompatibility complex (MHC) class II and intracellular adhesion mol. (ICAM-1) and the formation of some cytokines (tumor necrosis factor-alpha - TNF-.alpha.).

L9 ANSWER 57 OF 57 CAPLUS COPYRIGHT 2002 ACS
1996:451351 Document No. 125:126841 The clinical status of benzoporphyrin derivative. Levy, Julia G.; Chan, Agnes; Strong, H. Andrew (QLT PhotoTherapeutics Inc., Vancouver, BC, V6S 4H6, Can. . Proceedings of SPIE-The International Society for Optical Engineering, 2625(Photochemistry: Photodynamic Therapy and Other Modalities), 86-95 (English) 1996. CODEN: PSISDG. ISSN: 0277-786X. Publisher: SPIE The International Society for Optical Engineering.

AB Benzoporphyrin deriv. monoacid ring A (BPD) is currently in Phase II clin. trials for the **treatment** of cutaneous malignancies (basal cell carcinoma and cutaneous metastases) and psoriasis. Results to date suggest that this **photosensitizer** has potential in both of these areas. Recently, a clin. trial with BPD was initiated for the **treatment** of **age related macular degeneration**, a neovascular condition in the eye which leads to blindness. BPD is a lipophilic **photosensitizer** which is rapidly taken up by activated cells and the vascular endothelium of

neovasculature. The PDT effects seen with BPD appear to be a combination of vasculature occlusion and direct killing of target cells. Since many diseases involve either activated cells and/or neovasculature, PDT with **photosensitizers** with characteristics like those of BPD, has applications far wider that incol. A new area of interest involving **photosensitizers** is that of immune modulation. A no. of **photosensitizers** have been shown to effect immune modulation in animal models of immune dysfunction including autoimmunity (rheumatoid arthritis, lupus), cutaneous hypersensitivity and allografts. BPD and Photofrin have both been shown to be effective in ameliorating arthritic symptoms in a no. of animal models. The mechanisms by which immune modulation is effected in these studies still remains to be resolved.

L9 ANSWER 51 OF 51 BIOSIS COPYRIGHT 2001 BIOLOGICAL ABSTRACTS INC.
1995:446721 Document No.: PREV199548451021. Feasibility of laser targeted photo-occlusion of ocular vessels. Asrani, Sanjay; Zeimer, Ran (1). (1) Johns Hopkins Univ., Wilmer Ophthalmol. Inst., 600 N. Wolfe St., Wilmer Woods Room 311, Baltimore, MD 21287-9131 USA. British Journal of Ophthalmology, (1995) Vol. 79, No. 8, pp. 766-771. ISSN: 0007-1161.
Language: English.

AB Aims/Background: Neovascularisation occurs in many major ocular diseases such as diabetes, **age-related macular degeneration**, and sickle cell disease. Laser photocoagulation is typically used to obliterate the vessels but it also causes severe damage to adjacent normal tissues. This is a very significant limitation especially in the **treatment** of choroidal neovascularisation which often occurs large areas of the posterior pole and the fovea. A method, laser targeted delivery, has been developed capable of releasing drugs locally and non-invasively in the choroidal or retinal vasculature. This method could be used to target a photosensitiser to neovascular membranes and cause their selective occlusion by irradiating them. The targeting properties of the method promise to yield a **treatment** for neovascularisation that does not damage adjacent tissues and thus preserves vision. The purpose of the present study was to test the feasibility of occluding ocular vessels with this method. Method: The iris vessels of the albino rat were chosen because the **treatment** could be assessed unequivocally and followed with time. Aluminium phthalocyanine tetrasulphonate was encapsulated in heat sensitive liposomes and administered systemically. The iris vessels were irradiated with a yellow laser to raise their temperature to 41 degrees C, cause a phase transition in the liposomes and thereby locally release the photosensitiser. The laser was also used to excite the released photosensitiser and cause occlusion. The effect was monitored immediately and for 5 months thereafter. Controls for the effect of the laser and the unencapsulated drug were conducted. Results: The results demonstrated that occlusion can be achieved and sustained for the period of follow up. The controls showed that the effect was not due to heat or to the activation of the low dose of free drug. Conclusion: These preliminary findings indicate that laser targeted photo-occlusion is a promising new method for the **treatment** of neovascularisation.

=> d his

(FILE 'HOME' ENTERED AT 15:04:05 ON 06 DEC 2002)

FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 15:04:25 ON 06 DEC 2002
L1 11 S CHOROIDAL NEOVASCULATURE
L2 7 S L1 AND TREATMENT
L3 5 DUP REMOVE L2 (2 DUPLICATES REMOVED)
L4 16614 S MACULAR DEGENERATION
L5 3855 S L4 AND TREATMENT

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L6              0 S L5 AND ANTI-ANGIOSTATIN
L7           2846 S L5 AND AGE RELATED
L8             95 S L7 AND PHOTOSENSITIZER
L9             54 DUP REMOVE L8 (37 DUPLICATES REMOVED)

=> s l7 and angiostatin
L10            10 L7 AND ANGIOSTATIN

=> dup remove l11
PROCESSING COMPLETED FOR L10
L11             6 DUP REMOVE L1  (4 DUPLICATES REMOVED)

=> d l11 1-6 ibib abs

L11  ANSWER 1 OF 6  CAPLUS  COPYRIGHT 2002 ACS
2002:449443  Document No. 137:33918  Preparation of pyrimidinylaminothiazoles
     as tyrosine kinase inhibitors..  Bilodeau, Mark T.; Hartman, George D.;
     Hoffman, Carol M., Jr.; Rhodes, William C., Jr.; Manley, Peter J.; Rodman,
     Leonard; Fisk, John T.; Smith, Anthony M.; Tucker, Thomas J. (Merck &
     Co., Inc., USA).  PCT Int. Appl. WO 2002045652 A1  20020613, 169 pp.
     DESIGNATED STATES: W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ,
     CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE,
     GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT,
     LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU,
     SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA,
     ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW:  AT, BE, BF, BJ, CF, CG,
     CH, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG.  (English).  CODEN: PIXXD2.  APPLICATION:
     WO 2001-US44118 20011130.  PRIORITY: US 2000-PV251066 20001204.
GI
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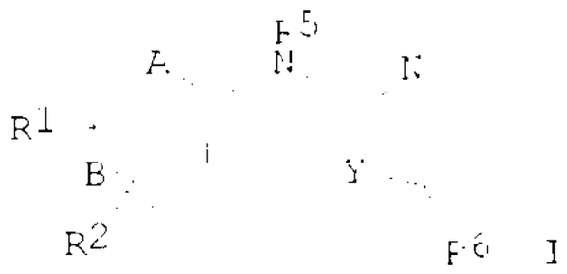

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AB   Title compds. [I; A, B = N, NO; Y = O, S, NR4; R1, R2 = H,
     perfluoroalkoxy, OH, cyano, halo, (substituted) alkyl(oxy)(carbonyl),
     aryl(oxy) carbonyl, heterocyclyl, etc.; R4 = H, aryl, alkyl; R5 = H,
     SO2R3, COR3, R3, NR3R3; R6 = aryl, cyano, halo, substituted alkyl,
     alkenyl, alkynyl, heterocyclyl, aminocarbonyl; R3 = alkyl, acyl,
     heterocyclyl], were prepd. for treating angiogenesis, cancer, tumor
     growth, atherosclerosis, age related macular
     degeneration, diabetic retinopathy, inflammation, etc.  Thus,
     4 aminopyrimidine was stirred with NaH in THF; 2 bromo-5-phenylthiazole
     was added and the mixt. was refluxed overnight to give
     5-phenylthiazol-2 yl pyrimidin-4-yl amine.  I inhibited vascular
     endothelial growth factor-stimulated mitogenesis of human vascular
     endothelial cells with IC50 = 0.01-5.0 nM.

L11  ANSWER 2 OF 6      MEDLINE                                  DUPLICATE 1
2002341368  Document Number: 22038186.      PubMed ID: 12072560.
     Adeno-associated virus type-2 expression of pigmented epithelium-derived
     factor or Kringles 1-3 of angiostatin reduce retinal
     neovascularization. Kaisler Brian J; Berns Kenneth I; Grant Maria B;
     Beliaev Denis; Hanneworth William W.  Department of Ophthalmology, Box
     1 (284, University of Florida, Gainesville, FL 3.610-0284, USA. )
     PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF
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AMERICA, (2002 Jun 25) 99 (13) 9309-14. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Neovascular diseases of the retina include **age-related macular degeneration** and diabetic retinopathy, and together they comprise the leading causes of adult-onset blindness in developed countries. Current surgical, pharmaceutical, and laser therapies for **age-related macular degeneration** (AMD) rarely result in improved vision, do not significantly prevent neovascularization (NV), and often result in at least some vision loss. To address this therapeutic gap, we determined the efficacy of recombinant adeno-associated viral (rAAV) serotype-2-mediated expression of pigment epithelium-derived factor (PEDF) or kringle domains 1-3 of **angiostatin** (K1K3) in reducing aberrant vessel formation in a mouse model of ischemia-induced retinal NV. Both PEDF and K1K3 are potent inhibitors of NV when injected directly, hence expression of these therapeutic factors from rAAV may provide long-term protection from neovascular eye disease. rAAV vectors expressing the therapeutic gene were injected into the eye of postnatal day 6 (P6) newborn mouse pups. Retinal NV was induced in P7 mice by exposure to elevated oxygen for 5 days followed by room air for another five days. Retinal NV was quantified by the number of vascular-endothelial-cell nuclei above the inner-limiting membrane in P12 eyes. The number of such vascular endothelial cell nuclei in eyes treated with rAAV-PEDF or rAAV-K1K3 was significantly reduced (both $P < 0.001$; $n = 12$) compared with control eyes. Ocular protein levels detected by ELISA correlate well with the reduction in NV and confirm that expression of antineovascular agents from rAAV vectors may be a therapeutically useful **treatment** of retinal and choroidal neovascular disease.

L11 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2002 ACS

2001:[illegible] Document No. 135:[illegible] Method and compositions for treating condition of the eye. Miller, Joan W.; Gragoudas, Evangelos S.; Kenney, Keven C. Massachusetts Eye and Ear Infirmary, USA). PCT Int. Appl. WO [illegible] A2. 20.08[illegible], 46 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG. English. CODEN: PIXXD2. APPLICATION: WO 2001-US41[illegible]. 20010[illegible]9. PRIORITY: US 2000-PV[illegible]1641 [illegible] 00 [illegible].

AB Provided are methods and compns. for the photodynamic therapy (PDT) of ocular conditions characterized by the presence of unwanted choroidal neovasculature, for example, neovascular **age-related macular degeneration.** The selectivity and sensitivity of the PDT method can be enhanced by combining the PDT with an anti-angiogenesis factor, for example, **angiostatin** or endostatin, or with an apoptosis-mediating factor. Furthermore, the selectivity and sensitivity of the PDT may be further enhanced by coupling a targeting moiety to the photosensitizer so as to target the photosensitizer to choroidal neovasculature.

L11 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2002 ACS

2001:[illegible] Document No. 134:[illegible] Preparation of 3-(3-indolyl)quinoline-2-one derivatives as tyrosine kinase inhibitors. Arrington, Kenneth L.; Balcarcel, Mark T.; Fraley, Mark E.; Hartman, George D.; Hoffman, William F.; Hungate, Randall W.; Kim, Yuntae (Merck & Co., Inc., USA). PCT Int. Appl. WO 2001029025 A2 20[illegible]0416, 150 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX,

MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US28629 20001016. PRIORITY: US 1999-PV160356 19991019.

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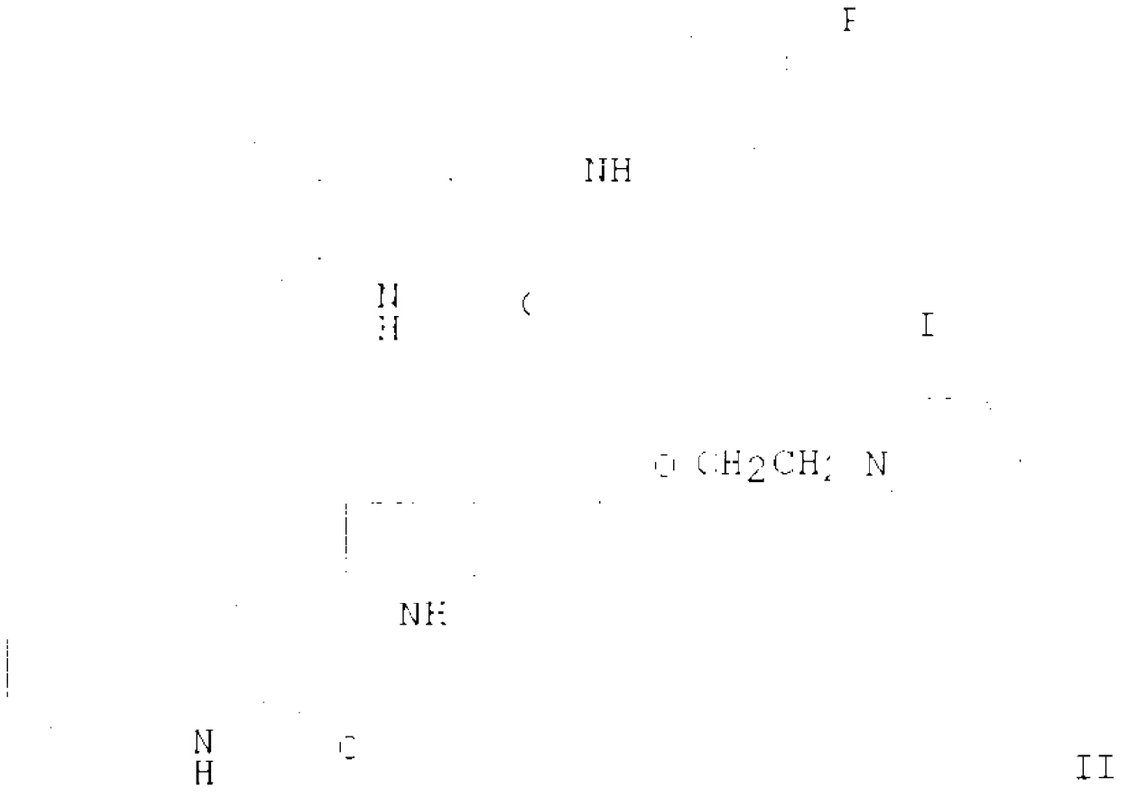

AB Title compds. [I; R = (CH3)2NCH2CH(CH3)CH2O, CH3OCH(CH2)·C6H5CH2)NCH2CH2O, CH3CH2)2NCH2CH2O, (CH3)(C6H5CH2)NCH2CH2CH2O, CH3OCH2CH2)2NCH2CH2NCH2CH2O, (CH3O)CH2CH2)(CH3SO2)NCH2, cycloalkylaminoalkyl, heterocyclylalkyl, etc.], stereoisomer, and pharmaceutically acceptable salts are prepd. and inhibit, regulate and/or modulate tyrosine kinase signal transduction. Title compds. are tested on VEGF-stimulated mitogenesis of human vascular endothelial cells in culture with IC50 values between 0.01-5.0 .mu.M. Pharmaceutical comprns. and methods of using them to treat tyrosine kinase-dependent diseases and conditions, such as angiogenesis, cancer, tumor growth, atherosclerosis, **age related macular degeneration**, diabetic retinopathy, inflammatory diseases, etc. are discussed. Thus, the title compd. II was prepd.

L11 ANSWER 5 OF 6 SCISEARCH COPYRIGHT 2002 ISI (R)

2001:719503 The Genuine Article (R) Number: 448XM. Inhibition of choroidal neovascularization by intravenous injection of adenoviral vectors expressing secretable endostatin. Mori K; Ando A; Gehlbach P; Nesbitt D; Takahashi K; Goldsteen D; Penn M; Chen C T; Mori K; Melia M; Phipps S; Moffat D; Brazzell R; Liau G; Dixon K H; Campochiaro P A (Reprint). Johns Hopkins Univ, Sch Med, Dept Ophthalmol, Maumenee 719, 600 N Wolfe St, Baltimore, MD 21287 USA (Reprint ; Johns Hopkins Univ, Sch Med, Dept Ophthalmol, Baltimore, MD 21287 USA; Johns Hopkins Univ, Sch Med, Dept Neurosci, Baltimore, MD 21287 USA; Genet Therapy, Gaithersburg, MD USA. AMERICAN JOURNAL OF PATHOLOGY (JUL 2001 Vol. 159, No. 1, pp. 313-320. Publisher: AMER SOC INVESTIGATIVE PATHOLOGY, INC. 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3993 USA. ISSN: 0002-9440. Pub. country: USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Endostatin is a cleavage product of collagen XVIII that inhibits tumor angiogenesis and growth. Interferon alpha 2a blocks tumor angiogenesis and

causes regression of hemangiomas, but has no effect on choroidal neovascularization (CNV). Therefore, inhibitors of tumor angiogenesis do not necessarily inhibit ocular neovascularization. In this study, we used an intravenous injection of adenoviral vectors containing a sigmEndo transgene consisting of murine immunoglobulin kappa -chain leader sequence coupled to sequence coding for murine endostatin to investigate the effect of high serum levels of endostatin on CNV in mice. Mice injected with a construct in which sig-mEndo expression was driven by the Rous sarcoma virus promoter had moderately high serum levels of endostatin and significantly smaller CNV lesions at sites of laser-induced rupture of Bruch's membrane than mice injected with null vector. Mice injected with a construct in which sig-mEndo was driven by the simian cytomegalovirus promoter had similar to 10-fold higher endostatin serum levels and had nearly complete prevention of CNV. There was a strong inverse correlation between endostatin serum level and area of CNV. This study provides proof of principle that gene therapy to increase levels of endostatin can prevent the development of CNV and may provide a new **treatment** for the leading cause of severe loss of vision in patients with **age-related macular degeneration.**

L11 ANSWER 6 OF 6 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
20002:7942 EMBASE AR-941. Oncolytic antiprostatic **treatment** of **age-related macular degeneration**
angiogenesis inhibitor. Scribera L.A.; Castaner R.M.; Leeson P.A., L.A. Scribera, Ergus Science, P.O. Box 540, 08080 Barcelona, Spain. Drugs of the Future 25/6 (551-557) 2001.
Date: 26.
ISSN: 0377-8282. CODEN: DRFUD4. Pub. Country: Spain. Language: English.
Summary Language: English.
AB Standardized shark cartilage liquid extract comprises the 0-500 kDa molecular fraction..

=> s lutetium texaphyrin
L12 131 LUTETIUM TEXAPHYRIN

=> s l12 and Lu-Tex
L13 4 L12 AND LU-TEX

=> s l12 and photosensitizer
L14 40 L12 AND PHOTOSENSITIZER

=> s l14 and anti-angiogenesis
L15 0 L14 AND ANTI-ANGIOGENESIS

=> s l14 and occlusion
L16 7 L14 AND OCCLUSION

=> s l14 and macular degeneration
L17 6 L14 AND MACULAR DEGENERATION

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L18 ANSWER 1 OF 5 SCISEARCH COPYRIGHT 2002 ISI (R)
2002:684119 The Genuine Article (R) Number: 583XT. CME photodynamic therapy for choroidal neovascularization - A review. Woodburn K W; Engelman C J; Blumenkranz M S (Reprint). Stanford Univ, Med Ctr, Dept Ophthalmol, Boswell A 157, Stanford, CA 94305 USA (Reprint); Stanford Univ, Med Ctr, Dept Ophthalmol, Stanford, CA 94305 USA. RETINA-THE JOURNAL OF RETINAL AND

VITREOUS DISEASES (AUG 2002) Vol. 22, No. 4, pp. 391-405. Publisher: LIPPINCOTT WILLIAMS & WILKINS. 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. ISSN: 0[illegible]-104X. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Purpose: To review the biophysical basis and current state of therapy for photodynamic closure of subfoveal choroidal neovascularization in the eye.

Methods: A review of the literature is included, which encompasses the chemical structure, biophysical mechanism of action, range of available agents, status of clinical trials, clinical indications, results of treatments, complications, and future directions.

Results: Photodynamic therapy has been shown to be effective in closing both experimental choroidal neovascularization in animal models as well as subfoveal choroidal neovascularization in humans. The therapy results in temporary closure of choroidal new vessels for a period of approximately 1 to 4 weeks. By 12 weeks, most patients have reperfusion or reproliferation of choroidal new vessels resulting in the need for retreatment to achieve continued closure and visual stabilization. Differences exist in the quantum yield, clinical efficiency, and light and sensitizer dose requirements between different classes of agents. Further clinical trials will be required to determine the optimal form of therapy, with verteporfin (Visudyne) as the only currently approved agent. Other agents, including tin etiopurpurin (Purlytin) and motexafin lutetium (Optrin), are currently undergoing phase III, and phase II trials, respectively.

Conclusions: Photodynamic therapy is a promising treatment modality shown to be effective in achieving closure and stabilization of vision loss compared with placebo control in eyes with subfoveal choroidal neovascularization.

L18 ANSWER 2 OF 5 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
20028 2117 EMBASE Photodynamic therapy of age-related **macular degeneration**: History and principles. Van den berg H., H. Van den Bergh, Swiss Federal Inst. of Technology, EPFL-ENAC-LPAS, CH-1015 Lausanne, Switzerland. hubert.vandenbergh@epfl.ch. Seminars in Ophthalmology 16 4 181-200 2001.
Refs: 165.
ISSN: 0[illegible]-05[illegible]. CODEN: SEOPET. Pub. Country: Netherlands. Language: English. Summary Language: English.

AB We briefly review the history and principles of photodynamic therapy (PDT), especially as it is applied to choroidal neovascularization (CNV) in age-related **macular degeneration** (AMD). After a brief general history of PDT, we discuss the relationship between the physicochemical structure and photodynamic activity of the second-generation **photosensitizers**, such as those in current clinical use. We then discuss the basic photophysics of **photosensitizer** molecules, and describe the initial chemical reactions induced by activated sensitizers. We outline a novel method for screening **photosensitizers** to be used in treating CNV, as well as the complex intermolecular pathways modulated by PDT-induced oxidative stress and the vascular effects of PDT in solid tumors. The paper closes with a discussion of how all this information might be used to improve the selectivity and efficacy of initially useful **photosensitizers**.

L18 ANSWER 3 OF 5 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 1
2001:[illegible] The Genuine Article (R) Number: 441ET. Texaphyrins: a new approach to drug development. Mody T D (Reprint); Sessler J L. Pharmacyclics Inc, [illegible] E Arques Ave, Sunnyvale, CA 94085 USA (Reprint); Pharmacyclics Inc, Sunnyvale, CA 94085 USA; Univ Texas, Dept Chem & Biochem, Austin, TX 78712 USA. JOURNAL OF PORPHYRINS AND PHTHALOCYANINES (FEB 2001) Vol. 5, No. 2, pp. 134-142. Publisher: JOHN WILEY & SONS LTD. BAFFINS LANE CHICHESTER, W SUSSEX PO19 1UD, ENGLAND. ISSN: 1088-4246. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The texaphyrins are prototypical metal-coordinating expanded porphyrins. They represent a burgeoning class of pharmacological agents that show promise for an array of medical applications. Currently, two different water-soluble lanthanide texaphyrins, namely motexafin gadolinium (Gd-Tex, .) and motexafin lutetium (**Lu-Tex**,), are involved in multi-center clinical trials for a variety of indications. The first of these agents, XCYTRIN(R) (motexafin gadolinium Injection, is being evaluated as a potential X-ray radiation enhancer in a randomized Phase III clinical trial in patients with brain metastases. The second, in various formulations, is being evaluated as a **photosensitizer** for use in: i) the photodynamic treatment of recurrent breast cancer (LUTRIN(R) Injection; now in Phase IIb clinical trials); ii) photoangioplastic reduction of atherosclerosis involving peripheral and coronary arteries (ANTRIN(R) Injection; now in Phase II and Phase I clinical trials, respectively; and iii) light-based age-related **macular degeneration** (OPTRIN(R) Injection; currently under Phase II clinical evaluation), a vision-threatening disease of the retina. In this article, these developments, along with fundamental aspects of the underlying chemistry are reviewed. Copyright (C) 2001 John Wiley & Sons, Ltd.

L18 ANSWER 4 OF 5 SCISEARCH COPYRIGHT 2004 ISI (R)
2000:[illegible] The Genuine Article (R) Number: [illegible]LH. Photodynamic therapy using **Lu-Tex** induces apoptosis in vitro, and its effect is potentiated by angiostatin in retinal capillary endothelial cells. Penno E B; Debiec F C; Hilmer S A; Gragoudas E S; Miller J W (reprint). HARVARD UNIV, MASSACHUSETTS EYE & EAR INFIRM, OCH MED, RETINA SERV, LASER LAB, 243 CHARLES ST, BOSTON, MA 02114 (Reprint); HARVARD UNIV, MASSACHUSETTS EYE & EAR INFIRM, OCH MED, RETINA SERV, LASER LAB, BOSTON, MA 02114; HARVARD UNIV, SCHEPENS EYE RES INST, OCH MED, BOSTON, MA 02114. INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE (NOV 2000) Vol. 41, No. 12, pp. 3963-3971. Publisher: ASSOC RESEARCH VISION OPHTHALMOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998. ISSN: 0146-0404. Pub. Country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB PURPOSE. To examine the effect of combining angiostatin with photodynamic therapy (PDT) using **Lutetium Texaphyrin** (**Lu-Tex**; Alcon, Fort Worth, TX) as a **photosensitizer** in bovine retinal capillary endothelial (BRCE) and retinal pigment epithelial (RPE) cells and to determine the mode of PDT-induced cell death in these cell lines.

METHODS. Cultured BRCE and RPE cells were incubated with angiostatin (50 ng/ml) for 12 hours and subjected to **Lu-Tex**/PDT, using treatment parameters previously optimized (3 microg/ml **Lu-Tex** for 3 minutes followed by timed irradiation at 732 nm). Cellular survival was assessed after a 1-week cellular proliferation. Data were analyzed using Student's t-test. Caspase 3 activity was monitored in cells after PDT using a fluorogenic substrate, (Asp-Glu-Val-Asp)-AFC (7-amino-4-trifluoromethyl coumarin) [DEVD-AFC], of caspase 3. After PDT, expression of Bcl-2, Bcl-X-L, Bax, and Bak was also examined in cell lysates by Western blot analysis.

RESULTS. A synergistic cytotoxic effect of angiostatin and **Lu-Tex** PDT was observed in BRCE cells at all fluences used (5, 10, and 20 J/cm(2); P less than or equal to .05). These findings applied only if angiostatin was delivered before PDT. No such interactive killing effect was observed in RPE cells. Caspase 3 activity was elevated within 15 minutes of PDT in BRCE and RPE cells and was fluence dependent. Differential modulation of Bcl-2 family members was observed after PDT in BRCE and RPE cells.

CONCLUSIONS. The combination of angiostatin and **Lu-Tex** PDT potentiates the cytotoxic effect of **Lu-Tex**/PDT on BRCE but not on RPE cells. This may provide a strategy to increase the selectivity of PDT in damaging capillary endothelial cells

with less damage to RPE cells. **Lu-Tex**/PDT induces rapid caspase-dependent apoptosis in BRCE and RPE cells. Furthermore, **Lu-Tex** PDT induces apoptosis through selective modulation of members of the Bcl-2 family and differs between BRCE and RPE cells.

L18 ANSWER 9 OF 9 SCISEARCH COPYRIGHT 2002 ISI (R)
2000:195997 The Genuine Article (R) Number: 285YZ. Texaphyrins - New drugs with diverse clinical applications in radiation and photodynamic therapy. Sessler J L Reprint ; Miller R A. UNIV TEXAS, DEPT CHEM & BIOCHEM, AUSTIN, TX 78712 Reprints; PHARMACYCL INC, SUNNYVALE, CA 94086. BIOCHEMICAL PHARMACOLOGY (1 APR 2000) Vol. 59, No. 7, pp. 733-739. Publisher: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND. ISSN: 0006-2952. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The texaphyrins are pentadentate metal-coordinating expanded porphyrins. They constitute a new series of synthetic porphyrin analogues that show promise as drugs for use in a range of medical therapies. Currently, two different water-solubilized lanthanide (III) texaphyrin complexes, namely the gadolinium (III) and lutetium(III) derivatives 1. and 2, motexafin and **Lu-Tex**, respectively, are being tested clinically. The first of these, XCYTRIN TM , is in a pivotal Phase III clinical trial as a potential enhancer of radiation therapy for patients with metastatic tumors to the brain receiving whole brain radiation therapy. The second, in various formulations, is being tested as a **photosensitizer** for use in: (i) the photodynamic treatment of recurrent breast cancer (LUTRIN(TM); Phase II clinical trials complete), (ii) photoangioplastic ablation of atherosclerosis involving peripheral arteries (ANTRIN(TM); now in Phase II testing , and (iii) light-based treatment of age-related **macular degeneration** (OPTRIN(TM); currently in Phase I clinical trials , a vision-threatening disease of the retina. Taken in concert, these two metallotexaphyrins provide a powerful new class of experimental drugs whose diverse potential utility is abetted by a combination of well-optimized physical features, favorable tissue biodistribution characteristics, and novel mechanisms of action; Interestingly, these mechanisms may alter conventional wisdom regarding mechanisms of radiation therapy and the pathophysiology of atherosclerosis. BIOCHEM PHARMACOL 59(7):733-739, 2000. (C) 2000 Elsevier Science Inc.

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L19 ANSWER 1 OF 19 SCISEARCH COPYRIGHT 2002 ISI (R)
2002:694119 The Genuine Article (R) Number: 584XT. ONE photodynamic therapy for choroidal neovascularization - A review. Woodburn K W; Engelman C C; Blumenkranz M S Reprint). Stanford Univ, Med Ctr, Dept Ophthalmol, Bowell A 157, Stanford, CA 94305 USA (Reprint ; Stanford Univ, Med Ctr, Dept Ophthalmol, Stanford, CA 94305 USA. RETINA-THE JOURNAL OF RETINAL AND VITREOUS DISEASES (AUG 2002) Vol. 22, No. 4, pp. 391-405. Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. ISSN: 0275-004X. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Purpose: To review the biophysical basis and current state of therapy for photodynamic closure of subfoveal choroidal neovascularization in the eye.

Methods: A review of the literature is included, which encompasses the chemical structure, biophysical mechanism of action, range of available

agents, status of clinical trials, clinical indications, results of treatments, complications, and future directions.

Results: Photodynamic therapy has been shown to be effective in closing both experimental choroidal neovascularization in animal models as well as subfoveal choroidal neovascularization in humans. The therapy results in temporary closure of choroidal new vessels for a period of approximately 1 to 4 weeks. By 12 weeks, most patients have reperfusion or reproliferation of choroidal new vessels resulting in the need for retreatment to achieve continued closure and visual stabilization. Differences exist in the quantum yield, clinical efficiency, and light and sensitizer dose requirements between different classes of agents. Further clinical trials will be required to determine the optimal form of therapy, with verteporfin (Visudyne) as the only currently approved agent. Other agents, including tin etiopurpurin (Purlytin) and motexafin lutetium (Optrin), are currently undergoing phase III, and phase II trials, respectively.

Conclusions: Photodynamic therapy is a promising treatment modality shown to be effective in achieving closure and stabilization of vision loss compared with placebo control in eyes with subfoveal choroidal neovascularization.

L19 ANSWER 2 OF 19 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
20012..[illegible] EMBASE Review article: Photodynamic therapy and the alimentary tract. Selvaraj K.K.; Birbeck N.; McMillan T.; Wainwright M.; Walker I.T., A.J. Walker, BUPA Fylde Coast Hospital, St Walburgas Road, Blackpool, Lancashire, FY3 8BP, United Kingdom. normal.tibet@btinternet.com. Alimentary Pharmacology and Therapeutics [illegible] 2001.
Refs: [illegible].
ISSN: [illegible]. CODEN: APTHEN. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB Photodynamic therapy offers the possibility of relatively selective tumour necrosis and normal tissue healing. It has many potential applications but as yet no clear role. Articles, editorials and case reports published primarily in English and listed in Medline ISI up to April [illegible] or identified by a manual search have been reviewed in an attempt to provide a comprehensive overview of the use of photodynamic therapy in the alimentary tract. It is concluded that photodynamic therapy can be an effective treatment for superficial pre-malignant mucosal lesions and early cancers, especially in diffuse disease. Suitable patients include those wishing to avoid surgery, high risk surgerys or those in whom other forms of treatment have failed. Superiority over other methods of ablation has not so far been demonstrated. Cheaper and more effective **photosensitizers** and improved techniques of light delivery are likely to increase the application of photodynamic therapy.

L19 ANSWER 3 OF 19 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
20012 2214 EMBASE Photodynamic therapy in the canine prostate using motexafin lutetium. Hsi R.A.; Rapaport A.; Stranberg J.; Shu T.; Vulcan T.; Schinen. M.; Rodriguez C.; Chang J.; Saunders M.; Mason N.; Hahn S., R.A. Hsi, Department of Radiation Oncology, Virginia Mason Medical Center, CB-EC, 1100 Ninth Avenue, Seattle, WA 98101, United States. [illegible]@[illegible].org. Clinical Cancer Research [illegible] 2001.
Refs: [illegible].
ISSN: [illegible]. CODEN: CCREF4. Pub. Country: United States. Language: English. Summary Language: English.

AB Our purpose was to determine the feasibility of comprehensive treatment of the canine prostate with photodynamic therapy (PDT) using motexafin lutetium (**Lu-Tex**) and to evaluate the toxicity and tissue effects associated with this treatment. Twenty-five adult male beagles with normal prostate glands were given an i.v. injection of the second-generation **photosensitizer Lu-Tex** (2-6 mg/kg). An additional two dogs were used as controls and did not receive any photosensitizing drug. All 27 dogs underwent laparotomy to

expose the prostate. Three hours postinjection, a total dose of 75-150 J/cm of 732 nm laser light was delivered interstitially and/or transurethrally to the prostate via cylindrical diffusing fibers. Dogs were euthanized between 2 days and 3 months after PDT. All subjects were monitored for clinical evidence of toxicity. Specimens were examined macroscopically and microscopically to characterize the tissue reaction and assess extent of tissue effect as a result of treatment. Interstitial and/or transurethral PDT were successfully delivered in all dogs with no perioperative complications. No clinical evidence of acute urinary obstruction or rectal bleeding was noted. At all dose levels, macroscopic and microscopic evaluation revealed a prostatic tissue reaction characterized initially (within 48 h) by inflammation and necrosis followed by fibrosis and glandular epithelial atrophy. Comprehensive treatment of the entire prostate could be achieved using the interstitial alone approach or combined transurethral and interstitial approach. The transurethral alone approach did not result in complete coverage of the prostate. Dogs receiving transurethral or combined interstitial and transurethral treatment developed erythema and urethral epithelial disruption at all dose levels. Those receiving combined treatment at the highest dose level (LuTex 4 mg/kg, 150 J/cm light) developed urethral fistulae and peritonitis. Dogs treated with the interstitial alone approach were found to have the least amount of urethral damage. Comprehensive treatment of the canine prostate with LuTex PDT is feasible using an interstitial alone or combined interstitial and transurethral approach. The interstitial alone technique results in the least amount of toxicity. The prostatic tissue reaction to treatment is characterized by initial inflammation and necrosis followed by fibrosis and glandular epithelial atrophy.

L19 ANSWER 4 OF 19 EMBASE COPYRIGHT 2010 ELSEVIER SCI. B.V.
2001050660 EMBASE Photoangioplasty with local motexafin lutetium delivery reduces macrophages in a rabbit post-balloon injury model. Hayase M.; Woodburn K.W.; Perlroth J.; Miller R.A.; Baumgardner W.; York P.S.; Yeung A.. A. Yeung, Division of Cardiovascular Medicine, Stanford Univ. School of Medicine, 300 Pasteur Drive, Stanford, CA 94305, United States. alan_yeung@cvmed.stanford.edu. Cardiovascular Research 49 2 (449-455) 1 Feb 2001.
Refs: 26.
ISSN: 0008-6363. CODEN: CVREAU.
Publisher Ident.: S 0008-6363(00)00278-4. Pub. Country: Netherlands.
Language: English. Summary Language: English.
AB Objective: Motexafin lutetium (**Lu-Tex**, Antrin.RTM. Injection) is a **photosensitizer** that selectively accumulates in atheromatous plaque where it can be activated by far-red light. The localization and retention of intra-arterially administered **Lu-Tex** and its efficacy following activation by endovascularly delivered light (photoangioplasty) was evaluated. Methods: Bilateral iliac artery lesions were induced in 17 rabbits by balloon denudation, followed by a high cholesterol diet. **Lu-Tex** distribution within the atheroma was examined ex-vivo following local injection. Fluorescence spectral imaging and chemical extraction techniques were used to measure **Lu-Tex** levels within the atheroma and adjacent normal tissue. Photoactivation was performed 15 min following **Lu-Tex** administration (1.1 J/cm fiber at 200 mW/cm fiber). Two weeks post photoangioplasty, vessels were harvested and hematoxylin and eosin (H&E and RAM11 (macrophage) staining was performed. Results: Local delivery of **Lu-Tex** achieved immediate high concentrations within plaque (mean 40x control iliac atheroma). Mean percent plaque area in the treated segments was significantly lower than in the non-treated contralateral lesions (73 vs. 82%, P<0.01). No medial damage was observed. Quantitative analysis using RAM11 positive cells revealed significant reduction of macrophages in treated lesions in both the intima (9 vs. 22%, P<0.01) and in media (8 vs. 23%, P<0.01) compared

to untreated contralateral segments. Conclusions: Local delivery provides high levels of **Lu-Tex** selectively within atheroma. Photoactivation results in a significant decrease in macrophage and a small decrease in atheroma burden without damage to the normal vessel wall. .COPYRGT. 2001 Elsevier Science B.V.

L19 ANSWER 5 OF 19 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
2001[illegible]193 EMBASE Preclinical evaluation of motexafin lutetium-mediated intraperitoneal photodynamic therapy in a canine model. Griffin S.M.; Zhu T.; Solomenko M.; Del Piero F.; Kapatkin A.; Busch T.M.; Yodh A.; Polin G.; Baker T.; Flaker D.; Hahn S.M.. S.M. Hahn, Department of Radiation Oncology, University of Pennsylvania, 3400 Spruce Street, Philadelphia, PA 19104-4283, United States. hahn@xrt.upenn.edu. Clinical Cancer Research 7/2 (374 381.) 2001.
Refs: 24.
ISSN: 1078-0432. CODEN: CCREF4. Pub. Country: United States. Language: English. Summary Language: English.
AB Intraperitoneal photodynamic therapy (IP PDT) is an experimental cancer treatment in clinical development for the treatment of peritoneal carcinomatosis and sarcomatosis. A canine study of motexafin lutetium (**Lu-Tex**)-mediated IP PDT was performed to evaluate normal tissue toxicities of this treatment in the presence and absence of a bowel resection and to assess the feasibility of measuring **Lu-Tex** fluorescence in abdominal tissues. Thirteen dogs were treated with **Lu-Tex** (0.2-0.4 mg/kg i.v.) 3 h before laparotomy and 730 nm light delivery (fluences, 0.5-2.0 J/cm2; average fluence rate 150 mW/cm2). Laparoscopy was performed 7-10 days after the procedure to assess acute toxicities. In vivo fluorescence spectra were obtained from various abdominal tissues before and after light delivery using a fiber array probe with three source-detector distances. **Lu-Tex**-mediated IP PDT was well tolerated at the doses of drug and light studied. Bowel toxicity was not observed in animals treated with a bowel resection before PDT. Mild transient liver function test abnormalities without associated clinical sequelae were observed. No gross PDT-related abnormalities were observed at laparoscopy or necropsy; however, thickening in the glomerular capillary wall and the mesangium were noted microscopically in the kidneys of seven dogs. No renal function abnormalities were found. Analysis of the fluorescence spectra from intra-abdominal tissues suggests that measurements of **Lu-Tex** in situ are feasible and may provide a way of assessing **photosensitizer** concentration in vivo without the need for a biopsy. These results support the continued development of **Lu-Tex** as a candidate **photosensitizer** for IP PDT.

L19 ANSWER 6 OF 19 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
2002[illegible]637 EMBASE Photodynamic therapy of age-related macular degeneration: History and principles. Van den Bergh H., H. Van den Bergh, Swiss Federal Inst. of Technology, EPFL-ENAC-LPAS, CH-1015 Lausanne, Switzerland. hubert.vandenbergh@epfl.ch. Seminars in Ophthalmology 16/4 141-200 2001.
Refs: 165.
ISSN: 0882-0538. CODEN: SEOPE7. Pub. Country: Netherlands. Language: English. Summary Language: English.
AB We briefly review the history and principles of photodynamic therapy (PDT), especially as it is applied to choroidal neovascularization (CNV) in age-related macular degeneration (AMD). After a brief general history of PDT, we discuss the relationship between the physicochemical structure and photodynamic activity of the second-generation **photosensitizers**, such as those in current clinical use. We then discuss the basic photophysics of **photosensitizer** molecules, and describe the initial chemical reactions induced by activated sensitizers. We outline a novel method for screening **photosensitizers** to be used in treating CNV, as well as the complex biomolecular pathways

modulated by PDT-induced oxidative stress and the vascular effects of PDT in solid tumors. The paper closes with a discussion of how all this information might be used to improve the selectivity and efficacy of clinically useful **photosensitizers**.

L19 ANSWER 7 OF 19 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 1
2001:166714 The Genuine Article (R) Number: 403ET. Texaphyrins: a new approach to drug development. Magda T D Reprint ; Sessler J L. Pharmacyclics Inc, 995 E Arques Ave, Sunnyvale, CA 94085 USA (Reprint); Pharmacyclics Inc, Sunnyvale, CA 94086 USA; Univ Texas, Dept Chem & Biochem, Austin, TX 78712 USA. JOURNAL OF PORPHYRINS AND PHTHALOCYANINES (FEB 2001) Vol. 5, No. 2, pp. 194-198. Publisher: JOHN WILEY & SONS LTD. BAFFINS LANE CHICHESTER, W SUSSEX PO19 1UD, ENGLAND. ISSN: 1088-4246. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB The texaphyrins are prototypical metal-coordinating expanded porphyrins. They represent a burgeoning class of pharmacological agents that show promise for an array of medical applications. Currently, two different water-soluble lanthanide texaphyrins, namely motexafin gadolinium (**Gd-Tex**, 1) and motexafin lutetium (**Lu-Tex**, 2), are involved in multi-center clinical trials for a variety of indications. The first of these agents, XCYTRIN(R) motexafin gadolinium) Injection, is being evaluated as a potential X-ray radiation enhancer in a randomized Phase III clinical trial in patients with brain metastases. The second, in various formulations, is being evaluated as a **photosensitizer** for use in: (i) the photodynamic treatment of recurrent breast cancer (LUTRIN(R) Injection; now in Phase IIb clinical trials); (ii) photoangioplasty reduction of atherosclerosis involving peripheral and coronary arteries (ANTRIN(R) Injection; now in Phase II and Phase I clinical trials, respectively); and (iii) light-based age related macular degeneration (OPTRIN R Injection; currently under Phase II clinical evaluation), a vision-threatening disease of the retina. In this article, these developments, along with fundamental aspects of the underlying chemistry are reviewed. Copyright (C) 2001 John Wiley & Sons, Ltd.

L19 ANSWER 8 OF 19 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
2001367755 EMBASE **Photosensitizer** delivery for photodynamic therapy of choroidal neovascularization. Bernd E.Z.; Miller J.W., Dr. J.W. Miller, Angiogenesis Laboratory, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA, United States. jwmiller@meei.harvard.edu. Advanced Drug Delivery Reviews 51, 1 (67-79) 31 Oct 2001.
Refs: 92.
ISSN: 0169-409X. CODEN: ADDREE.
Publisher Ident.: S 0169-409X(01) 00195-7. Pub. Country: Netherlands.
Language: English. Summary Language: English.
AB The present review examines the importance of improving **photosensitizer** delivery for choroidal neovascularization (CNV) in light of the clinical impact of photodynamic therapy (PDT) for CNV. An overview of the classes of available **photosensitizers** is provided and the properties governing **photosensitizer** uptake and circulation in serum are discussed. Current delivery systems, for example liposomal formulations as well as the use of the promising strategy of antibody targeted delivery as a strategy to improve PDT selectivity and efficiency for CNV treatment are described. A summary of the work using verteporfin, tin ethyl purpurin and **Lu-Tex** - **photosensitizers** currently in clinical trials for CNV - is given.
COPYRIGHT. 2001 Elsevier Science B.V. All rights reserved.

L19 ANSWER 9 OF 19 MEDLINE DUPLICATE 2
2001025055 Document Number: 295-77.5. PubMed ID: 11053300. Photodynamic therapy using **Lu-Tex** induces apoptosis in vitro, and its effect is potentiated by angiostatin in retinal capillary endothelial

cells. Renno R Z; Delori F C; Holzer R A; Gragoudas E S; Miller J W. (Laser Laboratory, Retina Service, Massachusetts Eye and Ear Infirmary. Schepens Eye Research Institute, Harvard Medical School, Boston, USA.) INVESTIGATIVE OPHTHALMOLOGY AND VISUAL SCIENCE, (2000 Nov) 41 (12) 3962-71. Journal code: 7703701. ISSN: 0146-0404. Pub. Country: United States. Language: English.

AB PURPOSE: To examine the effect of combining angiostatin with photodynamic therapy (PDT) using **Lutetium Texaphyrin** (**Lu-Tex**; Alcon, Fort Worth, TX) as a **photosensitizer** in bovine retinal capillary endothelial (BRCE) and retinal pigment epithelial (RPE) cells and to determine the mode of PDT-induced cell death in these cell lines. METHODS: Cultured BRCE and RPE cells were incubated with angiostatin (50 microg/ml) for 18 hours and subjected to **Lu-Tex** PDT, using treatment parameters previously optimized (5 microgram/ml **Lu-Tex** for 30 minutes followed by timed irradiation at 732 nm). Cellular survival was assessed after a 1-week cellular proliferation. Data were analyzed using Student's t-test. Caspase 3 activity was monitored in cells after PDT using a fluorogenic substrate, (Asp-Glu-Val-Asp) (AFC) 7-amino-4-trifluoromethyl coumarin [DEVD-AFC], of caspase 3. After PDT, expression of Bcl-2, Bcl-xL, Bax, and Bak was also examined in cell lysates by Western blot analysis. RESULTS: A synergistic cytotoxic effect of angiostatin and **Lu-Tex** PDT was observed in BRCE cells at all fluences used (5, 10, and 20 J/cm(2); P < 0.05). These findings applied only if angiostatin was delivered before PDT. No such interactive killing effect was observed in RPE cells. Caspase 3 activity was elevated within 10 minutes of PDT in BRCE and RPE cells and was fluence dependent. Differential modulation of Bcl-2 family members was observed after PDT in BRCE and RPE cells. CONCLUSIONS: The combination of angiostatin and **Lu-Tex** PDT potentiates the cytotoxic effect of **Lu-Tex** PDT on BRCE but not on RPE cells. This may provide a strategy to increase the selectivity of PDT in damaging capillary endothelial cells with less damage to RPE cells. **Lu-Tex** PDT induces rapid caspase-dependent apoptosis in BRCE and RPE cells. Furthermore, **Lu-Tex** PDT induces apoptosis through selective modulation of members of the Bcl-2 family and differs between BRCE and RPE cells.

L19 ANSWER 18 OF 19 BIOSIS COPYRIGHT 2002 BIOS
2000:199497 Pre-Genuine Article (PA) Number: PA65VE. Texaphyrins - New drugs with diverse clinical applications in radiation and photodynamic therapy. Sessler J L (Reprint); Miller R A. UNIV TEXAS, DEPT CHEM & BIOCHEM, AUSTIN, TX 78712. (Reprint); PHARMACYCLICS INC, SUNNYVALE, CA 94086. BIOCHEMICAL PHARMACOLOGY, (1 APR 2000) Vol. 59, No. 7, pp. 733-739. Publisher: PERGAMON-ELSEVIER SCIENCE LTD. THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND. ISSN: 0006-2952. Pub. Country: USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ASCII AND IMAGE FORMATS

AB The texaphyrins are quintessential metal-coordinating expanded porphyrins. They constitute a new series of synthetic porphyrin analogues that show promise as drugs for use in a range of medical therapies. Currently, two different water-solubilized lanthanide(III) texaphyrin complexes, namely the gadolinium(III) and lutetium(III) derivatives 1 and 2 (Gd-Tex and **Lu-Tex**, respectively), are being tested clinically. The first of these, MOTEXIN(TM), is in a pivotal Phase III clinical trial as a potential enhancer of radiation therapy for patients with metastatic cancers to the brain receiving whole brain radiation therapy. The second, in various formulations, is being tested as a **photosensitizer** for use in: i) the photodynamic treatment of recurrent breast cancer (LUTRIN(TM); Phase II clinical trial complete), ii) photoangioplastic reduction of atherosclerosis involving peripheral arteries (ANTRIN(TM); now in Phase II testing), and (iii) light-based treatment of age-related macular degeneration (OPTRIN(TM); currently in Phase I clinical trials), a vision-threatening disease of the retina.

Taken in concert, these two metallotexaphyrins provide a powerful new class of experimental drugs whose diverse potential utility is abetted by a combination of well-optimized physical features, favorable tissue biolocalization characteristics, and novel mechanisms of action; interestingly, these mechanisms may alter conventional wisdom regarding mechanisms of radiation therapy and the pathophysiology of atherosclerosis. BIOCHEM PHARMACOL 60;7:733-749, 2000.

L19 ANSWER 11 OF 19 MEDLINE DUPLICATE 3
2000170754 Document Number: 20170754. PubMed ID: 10704551.

Lutetium texaphyrin (Lu-Tex): a potential new agent for ocular fundus angiography and photodynamic therapy. Blumenkranz M S; Woodburn K W; Qing F; Verdooner S; Hessel D; Miller B. (Pharmacyclics Inc, Sunnyvale, CA, USA.. ms.msb@forsythe.stanford.edu) . AMERICAN JOURNAL OF OPHTHALMOLOGY, (2000 Mar) 129 (3) 353-62. Journal code: 0370500. ISSN: 0002-9394. Pub. Country: United States. Language: English.

AB PURPOSE: To investigate the suitability of **lutetium texaphyrin (lu-tex)** as a fluorescence imaging agent in the delineation of retinal vascular and choroidal vascular diseases. The utilization of an efficient fluorescent molecule that is also a **photosensitizer** represents a unique opportunity to couple diagnosis and therapy. METHODS: Fundus fluorescence angiography comparing **lu-tex** (motexafin lutetium, Optrin, Pharmacyclics Inc, Sunnyvale, California) with the conventional angiographic dyes, sodium fluorescein, and indocyanine green (ICG), was performed on the eyes of normal and laser-injured New Zealand white rabbits. Plasma pharmacokinetic data and plasma protein binding were assessed in addition to light microscopy of the retina in both intact and laser-injured eyes.RESULTS: Normal retinal and choroidal vasculature was well delineated by **lu-tex** angiography. Experimentally induced choroidal and retinal vascular lesions were enhanced by **lu-tex** and demonstrated different staining patterns than fluorescein or ICG, particularly at the margins of the lesions. **Lu-tex** cleared rapidly from the plasma, with 84.7% bound to the high-density lipoprotein (HDL) fraction while less was bound to the low-density lipoprotein (LDL) fraction. No evidence of retinal toxicity after dye administration was observed by either ophthalmoscopy and fundus photography or by light microscopy. CONCLUSION: **Lu-tex** angiography is a potentially valuable method for retinal vascular and choroidal vascular evaluation, and it has advantages over fluorescein and ICG angiography. The same agent could conceivably be used for both the identification of abnormal vasculature and subsequent photodynamic treatment.

L19 ANSWER 12 OF 19 BIOSIS COPYRIGHT (C) BIOLOGICAL ABSTRACTS INC.
2000:146866 Document No.: PREV200000246866. Subcellular photocytotoxicity of Photofrin-II and **lutetium texaphyrin** in cells in vitro. Liang, H.; Shin, D. S.; Lee, Y. E.; Nguyen, D. C.; Kasravi, S.; Do, T.; Ardestani, P.; Berns, M. W. (1). (1) Beckman Laser Institute and Medical Clinic, University of California, Irvine, 1002 Health Sciences Road East, Irvine, CA, 92715-1475 USA. Lasers in Medical Science, (2000) Vol. 15, No. 2, pp. 106-112. ISSN: 0268-8921. Language: English. Summary Language: English.

AB Three cell types including bovine pulmonary artery endothelium cells (CPAE), rat kangaroo kidney cells (PTK2 , and human larynx epidermoid carcinoma cells (Hep 2) were used to study subcellular localisation and phototoxicity of Photofrin-II and **lutetium texaphyrin** (**Lu Tex**). Cells were examined for fluorescence after administration of the photosensitisers. Subcellular regions were exposed with a laser microbeam system that used an argon ion laser pumped dye laser generating a 630 nm for Photofrin-II and 730 nm for **Lu**

Tex. Fluorescence detection suggests that the Photofrin-II is bound primarily to the mitochondria with some diffuse fluorescence in the rest of the cytoplasm. The fluorescence in **Lu Tex** treated cells appears to be localised to the lysosomes. The percentage of damaged cells following light exposure to the different subcellular regions after Photofrin-II or **Lu Tex** treatment demonstrates that the nuclear region was the most sensitive target followed by the perinuclear region and peripheral cytoplasm region.

L19 ANSWER 13 OF 19 MEDLINE DUPLICATE 4
20010218[illegible] Document Number: 20[illegible]. PubMed ID: 1[illegible]. Fluorescence pharmacokinetics of **Lutetium Texaphyrin** (PCI-0[illegible], **Lu Tex**) in the skin and in healthy and tumoral hamster cheek pouch mucosa. Bellnier M; Padu A; Monnier P; van den Bergh H; Wagnieres G. (Institute of Environmental Engineering, DGR-LPAS, EPFL, Lausanne, Switzerland.) JOURNAL OF PHOTOCHEMISTRY AND PHOTOBIOLOGY. B, BIOLOGY, (2000 Mar) 55 (1) 5-[illegible]. Journal code: [illegible]. ISSN: 1011-1344. Pub. country: Switzerland. Language: English.

AB We have investigated the pharmacokinetics (PK) of **Lutetium Texaphyrin** (**Lu-Tex**), a second-generation **photosensitizer**, in the Syrian hamster cheek pouch early cancer model. Ten male hamsters, five with chemically induced early squamous cell cancer of the left cheek pouch, received an intracardiac injection of a 10 mg/ml **Lu-Tex** solution, resulting in a dose of 12 mg **Lu-Tex** per kg of bodyweight. The PK of the dye have been measured during the 24 h following the injection with an optical-fiber-based spectrofluorometer on the ventral skin, the healthy and the tumoral cheek-pouch mucosa. The **Lu-Tex** fluorescence is excited at 400 nm and detected around 740 nm. All the measurements yield very similar pharmacokinetic curves. The fluorescence intensity reaches a maximum between two and three hours after the injection and, at its maximum, it is consistently higher (up to 1.5 times) on the tumor than on the healthy mucosa. It remains smaller on the skin than on cheek-pouch mucosa. After 24 h, the **Lu-Tex** fluorescence is no longer detectable either on the skin, on the lesion or on the healthy mucosa. Moreover, **Lu-Tex** clearly displays a significant fluorescence selectivity between early carcinoma and healthy mucosa in this model. Furthermore, the inter-animal fluctuations of the fluorescence signal are small (+/-[illegible]) on the tumor-bearing mucosa. Eight-minute-long skin-irradiation tests have been performed 24 h after the injection of the **Lu-Tex** on the ventral skin of 20 additional animals with a solar simulator. No reaction is observed, either macroscopically or microscopically, which further demonstrates, as suggested by the fluorescence measurements, that this **photosensitizer** is significantly cleared from the skin after 24 h.

L19 ANSWER 14 OF 19 MEDLINE DUPLICATE 5
19992[illegible]79 Document Number: 99[illegible]. PubMed ID: [illegible]. Systemic application of **photosensitizers** in the chick chorioallantoic membrane (CAM) model: photodynamic response of CAM vessels and 5-aminolevulinic acid uptake kinetics by transplantable tumors. Hornung R; Hermann-Wilson M J; Kunel S; Liaw L H; Tadir Y; Berns M W. (Beckman Laser Institute and Medical Clinic, University of California, Irvine, USA.) JOURNAL OF PHOTOCHEMISTRY AND PHOTOBIOLOGY. B, BIOLOGY, (1999 Mar) 49 (1) 41-9. Journal code: [illegible]. ISSN: 1011-1344. Pub. country: Switzerland. Language: English.

AB The aim of this study is to modify the chick chorioallantoic membrane (CAM) model into a whole-animal tumor model for photodynamic therapy (PDT). By using intraperitoneal (i.p.) **photosensitizer** injection of the chick embryo, use of the CAM for PDT has been extended to include systemic delivery as well as topical application of **photosensitizers**. The model has been tested for its capability to

mimic an animal tumor model and to serve for PDT studies by measuring drug fluorescence and PDT-induced effects. Three second-generation **photosensitizers** have been tested for their ability to produce photodynamic response in the chick embryo CAM system when delivered by i.p. injection: 5-aminolevulinic acid (ALA), benzoporphyrin derivative monoacid ring A (BPD-MA), and **Lutetium texaphyrin** (**Lu-Tex**). Exposure of the CAM vasculature to the appropriate laser light results in light-dose dependent vascular damage with all three compounds. Localization of ALA following i.p. injections in embryos, whose CAMs have been implanted with rat ovarian cancer cells to produce nodules, is determined in real time by fluorescence of the photoactive metabolite protoporphyrin IX (PpIX). Dose-dependent fluorescence in the normal CAM vasculature and the tumor implants confirms the uptake of ALA from the peritoneum, systemic circulation of the drug, and its conversion to PpIX.

L19 ANSWER 15 OF 19 BIOSIS COPYRIGHT 2001 on STN DUPLICATE 6
1999:499599 The Genuine Article (R) Number: 240DK. Photosensitization by the near-IR-absorbing **photosensitizer lutetium texaphyrin**: Spectroscopic, in vitro and in vivo studies.
Kostenich G; Zaboronkova T; Lam A; Langzam Y; Malik Z; Orenstein A; Ehrenberg B (Reprint). BAR ILAN UNIV, DEPT PHYS, IL-52900 RAMAT GAN, ISRAEL (Reprint ; BAR ILAN UNIV, DEPT PHYS, IL-52900 RAMAT GAN, ISRAEL; CHAIM SHEBA MED CTR, DEPT PLAST SURG, IL-52621 TEL HASHOMER, ISRAEL; BAR ILAN UNIV, DEPT LIFE SCI, IL-52900 RAMAT GAN, ISRAEL. JOURNAL OF PORPHYRINS AND PHTHALOCYANINES, JUL-OCT 1998 Vol. 2, Nr. 4-5, pp. 333-340. Publisher: JOHN WILEY & SONS LTD, BAFFINS LANE CHICHESTER, W SUSSEX PO19 1UD, ENGLAND. ISSN: 1088-4246. Pub. country: ISRAEL. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND LALL FORMATS*

AB The spectroscopic and biological properties of the new **photosensitizer lutetium texaphyrin** (**Lu-Tex**) were assessed in vitro and in vivo on a C26 colon carcinoma model, in comparison with hematoporphyrin (Hp), photofrin II (PII) and chlorin e6 (Chl). Strong binding of **Lu-Tex** to lipid bilayer membranes was observed. The results of confocal fluorescence microscopy on C26 cells showed that **Lu-Tex** was localized in small vesicles in the cytoplasm, possibly in the lysosomes, while Chl and Hp were distributed in larger cytoplasmic vesicles attributed to mitochondria. Scanning electron microscopy and X-ray microanalysis revealed that photodynamic therapy with **Lu-Tex** induced only slight damage to the cell membrane, leading to a delayed cell response. Chl and Hp caused significant structural damage to the outer cell membrane, resulting in ionic imbalance and fast cell death. The in vitro quantitative assessment of the relative efficiency per absorbed photon of the sensitizers revealed that **Lu Tex** was less effective than Chl and Hp. However, the results of our in vivo study showed that at the same light and drug doses the anti-tumor efficiency of the agents was in the following order: **Lu-Tex** > Chl > PII. The strong in vivo anti-tumor effect of **Lu-Tex** can be explained by its higher integrated absorption in the long-wavelength range. © 1998 John Wiley & Sons, Ltd.

L19 ANSWER 16 OF 19 CAPLUS COPYRIGHT 2001 ACS
1998:338623 Document No. 129:158731 Enhancement of **lutetium texaphyrin** phototherapy with mitomycin C. Thiemann, Patricia; Woodburn, Kathryn W. (Pharmacyclics, Inc., Sunnyvale, CA, 94086, USA). Proceedings of SPIE-The International Society for Optical Engineering, 3247 (Optical Methods for Tumor Treatment and Detection: Mechanisms and Techniques in Photodynamic Therapy VII), 56-62 (English) 1998. CODEN: PSISDG. ISSN: 0277-786X. Publisher: SPIE-The International Society for Optical Engineering.

AB **Lutetium texaphyrin** (**Lu-Tex**) photodynamic therapy (PDT) relies on the presence of the water-sol.

Lu-Tex, oxygen, and light (activation around 730 nm). Cytotoxic oxygen species are produced that cause irreversible damage to biol. substrates. Damage maybe inflicted via direct cell kill mechanisms or through vasculature effects that cause hypoxia. The addn. of hypoxia enhanced drugs, such as Mitomycin C (MMC), can potentially increase the anti-tumor response. RIF-1 bearing C3H mice received 10 .mu.mol **Lu-Tex**/kg and were illuminated with 1.0 J/cm2 at 1 h postinjection. Mice received MMC (2.5 or 5 mg/kg, before and after light) in conjunction with PDT and were compared to subsets of drug alone controls. A significant improvement in PDT response was obsd. when MMC was added to the dosing regimen; the effect was more pronounced at the highest MMC dose of 5 mg/kg: MMC prior to PDT gave a median tumor regrowth time (10X original vol.) of 12 days compared to MMC and PDT alone, 10.3 and 14.9 days, resp. The anti-tumor activity of **lutetium texaphyrin** induced PDT was improved by the addn. of the bioreductive alkylating agent mitomycin C.

L19 ANSWER 17 OF 19 CAPLUS COPYRIGHT 2002 ACS
1998:583603 Document No. 129:181611 Photodynamic therapy trials with **lutetium texaphyrin** (**Lu-Tex**) in patients with locally recurrent breast cancer. Renschler, Markus F.; Yuen, Alan S.; Panella, Timothy J.; Wileman, T. Jeffrey; Dougherty, Shona; Esserman, Laura; Panjehpour, Masoud; Taber, Scott W.; Fingar, Victor H.; Lowe, Elizabeth; Engel, Julie B.; Lum, Bert; Woodburn, Kathryn W.; Cheong, Wei-Fung; Miller, Richard A. Pharmacyclics, Inc., Sunnyvale, CA, 94086, USA). Proceedings of SPIE-The International Society for Optical Engineering, 3247 Optical Methods for Tumor Treatment and Detection: Mechanisms and Techniques in Photodynamic Therapy VII , 38-30 (English) 1998. CODEN: PSISDG. ISSN: 1227-718X. Publisher: SPIE-The International Society for Optical Engineering.

AB Photodynamic therapy (PDT) of locally recurrent breast cancer has been limited to treatment of small lesions because of non-selective necrosis of adjacent normal tissues in the treatment field. **Lutetium Texaphyrin** PCI-0123, **Lu-Tex** is a **photosensitizer** with improved tumor localization that is activated by 732 nm light, which can penetrate through larger tumors. We have evaluated **Lu-Tex** in a Phase I trial and in an ongoing Phase II trial in women with locally recurrent breast cancer with large tumors who have failed radiation therapy. Patients received **Lu-Tex** i.v. by rapid infusion 3 h before illumination of cutaneous or s.c. lesions. In Phase I, **Lu Tex** doses were escalated from 0.6 to 7.1 mg/kg in 7 cohorts. 16 Patients with locally recurrent breast cancer lesions were treated. Dose limiting toxicities above 5.5 mg/kg were pain in the treatment field during therapy, and dysesthesias in light exposed areas. No necrosis of normal tissues in the treated field was noticed. Responses were obsd. in 60% of evaluable patients (n=15, 27% complete remission (CR), 33% partial remission (PR)), with 69% of lesions responding (n=73: 41% CR, 28% PR). In Phase II, 25 patients have been studied to date, receiving two treatments ranging from 1.1 to 5.0 mg/kg at a 24 h interval. Treatment fields up to 480 cm2 in size were treated successfully and activity has been obsd. Patients have experienced pain at the treatment site but no tissue necrosis. These studies demonstrate the feasibility of **Lu-Tex** PDT to large chest wall areas in women who have failed radiation therapy for the treatment of locally recurrent breast cancer. Treatment conditions are currently being optimized in the ongoing Phase II trials.

L19 ANSWER 18 OF 19 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1997:377378 Document No.: PREV199799676782. Photodynamic therapy trials with **lutetium texaphyrin** PCI-0123 **Lu-Tex**. Renschler, M. F. (1); Yuen, A. (1); Panella, T. J. (1); Wileman, T. J. (1); Julius, C.; Panjehpour, M.; Taber, S.; Fingar, V.; Horning, S.; Miller, R. A.; Lowe, E.; Engel, J.; Woodburn, K.; Young, S. W.. (1)

AB Photodynamic therapy (PDT) is a potentially selective treatment modality, which involves systemic administration of a **photosensitizer** dye. Dye accumulates in proliferating tissues such as tumors and neovascularization, followed by exposure of the photosensitized tissue to light at a wavelength at the absorption maximum of the dye. Excitation of the dye leads to photochemical damage of the targeted tissue. Various **photosensitizers** have been used in experimental **choroidal neovascularization** to investigate PDT. We have used benzoporphyrin derivative monoacid (BPD) and shown that it occludes experimental **choroidal neovascularization** CNV with no significant damage to the overlying neurosensory retina or underlying choroid. Clinical trials of PDT using BPD for exudative age-related macular degeneration (AMD) have started. Preliminary results suggest that CNV can be occluded in the early posttreatment phase, with some nonselective effects at high light doses. Further studies are underway to investigate whether PDT of AMD can help preserve long-term vision in patients.

L24 ANSWER 13 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1996:[illegible] Document No.: PREV[illegible]. Comparison study of **photosensitizer** uptake in retinal-choroidal liposomal benzoporphyrin derivative BPD. Kenney, E. B. [1]; Tolentino, M. J. [1]; Deboer, F. C.; Kim, R. Y. [1]; Ng, E. W. M. [1]; Canakis, C. S. [1]; **Gragoudas, E. S. (1); Miller, J. W. (1).** [1] Mass. Eye Ear Infirmary, Harvard Med. Sch., Boston, MA USA. Investigative Ophthalmology & Visual Science, (1996) Vol. 37, No. 3, pp. S797. Meeting Info.: 1996 Annual Meeting of the Association for Research in Vision and Ophthalmology Fort Lauderdale, Florida, USA April 21-26, 1996 ISSN: 0146-0404. Language: English.

L24 ANSWER 14 OF 15 MEDLINE
96182[illegible] Document Number: [illegible]. PubMed ID: 8600419. Liposomal benzoporphyrin derivative verteporfin photodynamic therapy. Selective treatment of **choroidal neovascularization** in monkeys.
Kramer M; **Miller J W;** Michaud N; Moulton R S; Hasan T; Flotte T J; **Gragoudas E S.** (Laser Research Laboratory, Retina Service, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, 02114, USA. OPHTHALMOLOGY, 1996 Mar 103 3) 427-38. Journal code: 7902443. ISSN: 0161-6420. Pub. country: United States. Language: English.

AB PURPOSE: The authors have previously shown that photodynamic therapy (PDT) using liposome-delivered benzoporphyrin derivative monoacid (BPD) effectively closed experimental **choroidal neovascularization** CNV. In the current study, the authors used a clinical preparation, liposomal BPD verteporfin in the same model, with experiments designed to establish optimal dye and light doses, and the timing of laser light irradiation after dye injection, for effective and selective closure of CNV. METHODS: Experimental CNV was induced in the maculae of cynomolgus monkeys. Liposomal BPD verteporfin was injected intravenously at doses of 1.0, 0.5, 0.375, and 0.25 mg/kg. Laser light at 692 nm then was applied to CNV, with an irradiance of 600 mW/cm2 and fluence of 150 J/cm2, at various times after dye injection, ranging from 5 to 115 minutes. Treatment effect was assessed by fundus photography and fluorescein angiography and confirmed by light and electron microscopy. The PDT of experimental CNV was studied to assess efficacy; PDT performance on normal eyes was studied to investigate selectivity. RESULTS: The CNV closure was demonstrated by fluorescein angiography and histopathologic findings at all tested dye doses. A dye dose of 0.375 mg/kg, with laser light irradiation applied 20 to 30 minutes after dye injection, optimized CNV closure with minimal retinal and choroidal damage. No major local adverse effects were noted, and the drug was well tolerated systematically. CONCLUSIONS: Liposomal BPD verteporfin is a potent **photosensitizer,** and PDT using this dye is a potentially effective and selective treatment for CNV.

L24 ANSWER 15 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1995:13244 Document No.: PREV199598246740. Imaging of experimental **choroidal neovascularization** (CNV) using liposomal benzoporphyrin derivative monoacid (BPD-MA) angiography. Kramer, M. (1); Kenney, A. G.; Delori, F.; Donnelly, E. T.; Hasan, D.; **Gragoudas, E. S.; Miller, J. W.**. (1) Mass. Eye Ear Infirmary, Boston, MA USA. Investigative Ophthalmology & Visual Science, (1995) Vol. 36, No. 4, Pt. 2, Suppl. Meeting Info.: Annual Meeting of the Investigative Ophthalmology and Visual Science Fort Lauderdale, Florida, USA May 14-19, 1995 ISSN: 1146-1404. Language: English.

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L25 1 L22 AND ANGIOSTATIN

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L26 1 DUP REMOVE L25 (0 DUPLICATES REMOVED)

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L26 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS
2001:547744 Document No. 135:149168 Methods and compositions for treating conditions of the eye. **Miller, Joan W.; Gragoudas, Evangelos S.; Renno, Reem Z.** Massachusetts Eye and Ear Infirmary, USA. PCT Int. Appl. WO 2001054686 A2 20010802, 46 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GH, IE, IT, LU, MC, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US41231 20010126. PRIORITY: US 2000-178441P 20000127.

AB Provided are methods and compns. for the photodynamic therapy (PDT) of ocular conditions characterized by the presence of unwanted choroidal neovascularization, for example, neovascular age related macular degeneration. The selectivity and sensitivity of the PDT method can be enhanced by combining the PDT with an anti-angiogenesis factor, for example, **angiostatin** or endostatin, and with an apoptosis-mediating factor. Furthermore, the selectivity and sensitivity of the PDT may be further enhanced by coupling a targeting moiety to the photosensitizer so as to target the photosensitizer to choroidal neovasculature.

L26 ANSWER 2 OF 2 BIOSEARCH COPYRIGHT 2002 IFI R
2000:[illegible] The Genuine Article (R) Number: [illegible]LH. Photodynamic therapy using Lu-Tex induces apoptosis in vitro, and its effect is potentiated by **angiostatin** in retinal capillary endothelial cells. **Renno R Z; Delori F C; Holtzer R A; Gragoudas E S; Miller J W (Reprint)**. HARVARD UNIV, MASSACHUSETTS EYE & EAR INFIRM, SCH MED, RETINA SERV, LASER LAB, 243 CHARLES ST, BOSTON, MA 02114 (Reprint); HARVARD UNIV, MASSACHUSETTS EYE & EAR INFIRM, SCH MED, RETINA SERV, LASER LAB, BOSTON, MA 02114; HARVARD UNIV, SCHEPENS EYE RES INST, SCH MED, BOSTON, MA 02114. INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE (NOV 2000) Vol. 41, No. 12, pp. 3965-3971. Publisher: ASSOC RESEARCH VISION OPHTHALMOLOGY INC. 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998. ISSN: 0146-0404. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB PURPOSE. To examine the effect of combining **angiostatin** with photodynamic therapy (PDT) using Lutetium Texaphyrin (Lu-Tex; Alcon, Fort Worth, TX) as a photosensitizer in bovine retinal capillary endothelial (BRCE) and retinal pigment epithelial (RPE) cells and to determine the

mode of PDT-induced cell death in these cell lines.

METHODS. Cultured BRCE and RPE cells were incubated with **angiostatin** (500 ng/ml) for 18 hours and subjected to Lu-Tex/PDT, using treatment parameters previously optimized (3 mug/ml Lu-Tex for 30 minutes followed by timed irradiation at 732 nm). Cellular survival was assessed after a 7-week cellular proliferation. Data were analyzed using Student's t-test. Caspase 3 activity was monitored in cells after PDT using a fluorogenic substrate, (Asp-Glu-Val-Asp)-AFC 7-amino-4-trifluoromethyl coumarin (DEVD-AFC), of caspase 3. After PDT, expression of Bcl-2, Bcl-X-L, Bax, and Bak was also examined in cell lysates by Western blot analysis.

RESULTS. A synergistic cytotoxic effect of **angiostatin** and Lu-Tex/PDT was observed in BRCE cells at all fluences used (5, 10, and 20 J/cm(2)); P less than or equal to 0.05). These findings applied only if **angiostatin** was delivered before PDT. No such interactive killing effect was observed in RPE cells. Caspase 3 activity was elevated within 15 minutes of PDT in BRCE and RPE cells and was fluence dependent. Differential modulation of Bcl-2 family members was observed after PDT in BRCE and RPE cells.

CONCLUSIONS. The combination of **angiostatin** and Lu-Tex PDT potentiates the cytotoxic effect of Lu-Tex/PDT on BRCE but not on RPE cells. This may provide a strategy to increase the selectivity of PDT in damaging capillary endothelial cells with less damage to RPE cells. Lu-Tex/PDT induces rapid caspase-dependent apoptosis in BRCE and RPE cells. Furthermore, Lu-Tex PDT induces apoptosis through selective modulation of members of the Bcl-2 family and differs between BRCE and RPE cells.

=> s l22 and azo dye
L27 1 L22 AND AZO DYE

=> d l27 cbib abs

L27 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS
2001:547738 Document No. 135:149263 Methods and compositions for treating condition of the eye. **Miller, Joan W.; Gragoudas, Evangelos S.; Renno, Reem Z.** Massachusetts Eye and Ear Infirmary, USA. PCT Int. Appl. WO 2001058440 A2 20010816, 46 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, GA, GN, DE, DK, EA, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. English. CODEN: PIXXD2. APPLICATION: WO 2001-US4231 20010209. PRIORITY: US 2000-PV182441 20000210.

AB Provided are methods and compns. for the photodynamic therapy (PDT) of ocular conditions characterized by the presence of unwanted choroidal neovasculature, for example, neovascular age-related macular degeneration. The selectivity and sensitivity of the PDT method can be enhanced by combining the PDT with an anti-angiogenesis factor, for example, angiostatin or endostatin, or with an apoptosis-modulating factor. Furthermore, the selectivity and sensitivity of the PDT may be further enhanced by coupling a targeting moiety to the photosensitizer so as to target the photosensitizer to choroidal neovasculature.

=> s l22 and xanthene derivative
L28 1 L22 AND XANTHENE DERIVATIVE

=> d l28 cbib abs

L28 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS
2001:59773 Document No. 135:149263 Methods and compositions for treating condition of the eye. **Miller, Joan W.; Gragoudas, Evangelos S.; Renno, Reem Z.** (Massachusetts Eye and Ear Infirmary, USA). PCT Int. Appl. WO 2001-US4131 A2 20010816, 46 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US4131 20010209. PRIORITY: US 2000-PV181441 20000210.

AB Provided are methods and compns. for the photodynamic therapy (PDT) of ocular conditions characterized by the presence of unwanted choroidal neovasculature, for example, neovascular age-related macular degeneration. The selectivity and sensitivity of the PDT method can be enhanced by combining the PDT with an anti-angiogenesis factor, for example, angiostatin or **endostatin**, or with an apoptosis-modulating factor. Furthermore, the selectivity and sensitivity of the PDT may be further enhanced by coupling a targeting moiety to the photosensitizer so as to target the photosensitizer to choroidal neovasculature.

=> s l12 and angiostatin
L29 0 L12 AND ANGIOSTATIN

=> s l12 and angiostatin
MISSING OPERATOR L12 AND
The search profile that was entered contains terms or nested terms that are not separated by a logical operator.

=> s l12 and endostatin
L30 1 L12 AND ENDOSTATIN

=> d l30 ibib abs

L30 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS
2001:59773 Document No. 135:149263 Methods and compositions for treating condition of the eye. **Miller, Joan W.; Gragoudas, Evangelos S.; Renno, Reem Z.** (Massachusetts Eye and Ear Infirmary, USA). PCT Int. Appl. WO 2001-US4131 A2 20010816, 46 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US4131 20010209. PRIORITY: US 2000-PV181441 20000210.

AB Provided are methods and compns. for the photodynamic therapy (PDT) of ocular conditions characterized by the presence of unwanted choroidal neovasculature, for example, neovascular age-related macular degeneration. The selectivity and sensitivity of the PDT method can be enhanced by combining the PDT with an anti-angiogenesis factor, for example, angiostatin or **endostatin**, or with an apoptosis-modulating factor. Furthermore, the selectivity and sensitivity of the PDT may be further enhanced by coupling a targeting moiety to the photosensitizer so as to target the photosensitizer to choroidal neovasculature.

Pharmacyclics Inc., Sunnyvale, CA USA. Photochemistry and Photobiology, (1997) Vol. 65, No. SPEC. ISSUE, pp. 47S-48S. Meeting Info.: 25th Annual Meeting of the American Society for Photobiology St. Louis, Missouri, USA July 5-10, 1997 ISSN: 0031-8655. Language: English.

L19 ANSWER 14 OF 19 MEDLINE DUPLICATE 7
97354099 Document Number: 97354099. PubMed ID: 9210320. In vivo photodynamic therapy with the new near-IR absorbing water soluble **photosensitizer lutetium texaphyrin** and a high intensity pulsed light delivery system. Rosenbach G; Orenstein A; Roitman L; Malik Z; Ehrenberg B. (Plastic Surgery Department, Sheba Medical Center, Tel Hashomer, Israel. JOURNAL OF PHOTOCHEMISTRY AND PHOTOBIOLOGY. B, BIOLOGY, (1997 May) 39 (1) 44-52. Journal code: 8804966. ISSN: 1011-1344. Pub. country: Switzerland. Language: English.

AB An in vivo fluorescence monitoring and photodynamic therapy (PDT) study was performed using the new **photosensitizer lutetium texaphyrin** (**Lu-Tex**). This **photosensitizer** is water soluble and has the additional advantage of strong absorption near 730 nm. C26 colon carcinoma was transplanted in the foot of BALB/c mice. In vivo fluorescence spectroscopy was applied to study **Lu-Tex** tissue distribution kinetics. For this purpose, fluorescence intensity both in the foot with the tumor and in the normal foot was measured in vivo by the laser induced fluorescence (LIF) system. For PDT, both feet of the mice were irradiated simultaneously with the use of a new high intensity pulsed light delivery system, the Photodyne. The results of the LIF measurements showed that the maximal fluorescence intensity ratio between the normal and tumor bearing foot (FIR) was observed 24-48 h after the agent injection. Photoirradiation with doses from 40 to 240 J cm-2 (0.6 J cm-2 per 2 ms pulse, 1 Hz) 24 h after injection of **Lu-Tex** at a dose of 2.5 mg kg-1 caused significant tumor necrosis and delay in the tumor growth rate. The antitumor effect was enhanced with increasing light doses. Normal tissue response to PDT with **Lu-Tex** was determined as the damage index of the normal foot, which was irradiated simultaneously with the tumor bearing foot. The normal tissue response after PDT with **Lu-Tex** was compared with 5-aminolevulinic acid (ALA) induced protoporphyrin IX (PP), chlorin e6 (Chl) and Photofrin (PII) at the same values of antitumor effect. The results showed that at 50, 60 and 100% inhibition of tumor growth the orders of the values of normal foot damage indexes were as follows: ALA > **Lu-Tex** > or = PII > Chl, PII > ALA > **Lu-Tex** > Chl and PII > **Lu-Tex** > ALA > Chl, respectively.

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=> s  miller p/au or gragoudas e/au or renno r?/au)
L20          43944 (MILLER P/AU OR GRAGOUDAS E?/AU OR RENNO R?/AU)

=> s l20 and choroidal ne vascular?
L21           151 L20 AND CHOROIDAL NEOVASCULAR?

=> dup remove l21
PROCESSING COMPLETED FOR L21
L22           117 DUP REMOVE L21 (78 DUPLICATES REMOVED)

=> s l22 and photosensitizer
L23            15 L22 AND PHOTOSENSITIZER

=> dup remove l23
PROCESSING COMPLETED FOR L23
L24            15 DUP REMOVE L23 (0 DUPLICATES REMOVED)

=> d l24 1-15 cbib abs
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L24 ANSWER 1 OF 15 MEDLINE
20023473?6 Document Number: 2?0?691. PubMed ID: 12091441. Verteporfin photodynamic therapy in the rat model of **choroidal neovascularization**: angiographic and histologic characterization. Jarrett David N; Ezra Eric; Tanada Yoshiko; Michaud Norman; Connolly Edward; **Gragoudas Evangelos S; Miller Joan W.** (Retina Service and Angiogenesis Laboratory, Massachusetts Eye and Ear Infirmary, Harvard Medical School, 243 Charles Street, Boston, MA 02114, USA.) INVESTIGATIVE OPHTHALMOLOGY AND VISUAL SCIENCE, 2002 Jul; 43 (7) 2384-91. Journal code: 7703701. ISSN: 0146-0404. Pub. country: United States. Language: English.

AB PURPOSE: To develop a model of verteporfin photodynamic therapy (PDT) for experimental **choroidal neovascularization** CNV in the rat. METHODS: A laser injury model was used to induce experimental CNV in rats. The transit and accumulation of the **photosensitizer** verteporfin was assessed angiographically in CNV lesions, to determine the optimal time for delivery of light energy. The CNV lesions were then treated with verteporfin PDT, with two doses of verteporfin (3.0 and 6.0 mg/kg) and four activating doses of light energy (12, 25, 50, and 100 J/cm(2)). Closure of the CNV was assessed both angiographically and histologically. Verteporfin PDT was also performed on areas of normal choroid and retina at the two verteporfin doses and four light energy doses. The effect of these treatments on these structures was also assessed angiographically and histologically. RESULTS: Peak verteporfin intensities in the CNV were detected at 15 to 20 minutes after intravenous injection. Rates of closure of the CNV varied as a function of the dose of verteporfin and of the activating light energy. Angiographic closure of the CNV correlated with damage to the neovascular complex, as seen with light and electron microscopy. Damage to areas of normal choroid and retina treated with verteporfin PDT also varied as a function of the verteporfin and light energy doses. CONCLUSIONS: Verteporfin PDT for experimental CNV in the rat is a feasible, effective, and reproducible model that can be used for testing the efficacy of adjunctive therapy to verteporfin PDT.

L24 ANSWER 2 OF 15 CAPLUS COPYRIGHT 2002 ACS
2001:5?7?3? Document No. 135:149?63 Methods and compositions for treating conditions of the eye. **Miller, Joan W.; Gragoudas, Evangelos S.; Renno, Reem Z.** (Massachusetts Eye and Ear Infirmary, USA). PCT Int. Appl. WO 2001?3?40 A2 20010426, 4? pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD?. APPLICATION: WO 2000-US?4231 20000?08. PRIORITY: US 1999-?161?41 19991020.

AB Provided are methods and compns. for the photodynamic therapy (PDT) of ocular conditions characterized by the presence of unwanted choroidal neovasculature, for example, neovascular age-related macular degeneration. The selectivity and sensitivity of the PDT method can be enhanced by combining the PDT with an anti-angiogenesis factor, for example, angiostatin or endostatin, or with an apoptosis-modulating factor. Furthermore, the selectivity and sensitivity of the PDT may be further enhanced by coupling a targeting moiety to the **photosensitizer** or as to target the **photosensitizer** to choroidal neovasculature.

L24 ANSWER 3 OF 15 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
2001?4?412 EMBASE Erratum: **Photosensitizer** delivery for photodynamic therapy of **choroidal neovascularization** (Advanced Drug Delivery Reviews (2001) 52 (61-78) PII: S0169409X01001958).

Renno R.Z.; Miller J.W.. Dr. J.W. Miller, Angiogenesis Laboratory, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA, United States. jwmiller@meei.harvard.edu. Advanced Drug Delivery Reviews 54,1 (31 3 Dec 2001.
ISSN: 0169-409X. CODEN: ADDREP.
Publisher Ident.: S 0169 409X(01)00238-1. Pub. Country: Netherlands.
Language: English.

L24 ANSWER 4 OF 19 CAPLUS COPYRIGHT 2001 ACS
2001:811911 Addendum to "**Photosensitizer** delivery for photodynamic therapy of **choroidal neovascularization**" [Adv. Drug Deliv. Rev. 52 (2001) 63-76]. **Renno, Reem Z.; Miller, Joan W.** Massachusetts Eye and Ear Infirmary, Angiogenesis Laboratory, Retina Service, Harvard Medical School, Boston, MA, USA). Advanced Drug Delivery Reviews, 54(1), 133 (English) 2001. CODEN: ADDREP. ISSN: 0169-409X. Publisher: Elsevier Science Ireland Ltd..
AB Unavailable

L24 ANSWER 5 OF 19 MEDLINE
2001564[illegible] Document Number: 21511295. PubMed ID: 11672876.
Photosensitizer delivery for photodynamic therapy of **choroidal neovascularization**. **Renno R Z; Miller J W.** (Retina Service, Angiogenesis Laboratory, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA, USA.) Adv Drug Deliv Rev, (2001 Oct 31) 52 (1) 63-76. Ref: 92. Journal code: 8710515. ISSN: 0169-409X. Pub. Country: Netherlands. Language: English.
AB The present review examines the importance of improving **photosensitizer** delivery for **choroidal neovascularization** (CNV) in light of the clinical impact of photodynamic therapy (PDT) for CNV. An overview of the classes of available **photosensitizers** is provided and the properties governing **photosensitizer** uptake and circulation in serum are discussed. Current delivery systems, for example liposomal formulations as well as the use of the promising strategy of antibody targeted delivery as a strategy to improve PDT selectivity and efficiency for CNV treatment are described. A summary of the work using Verteporfin, tin ethyl purpurin and Lu-Tex- **photosensitizers** currently in clinical trials for CNV is given.

L24 ANSWER 6 OF 19 BIOSIS COPYRIGHT 2001 BIOS
2000:[illegible] The Genuine Article (R) Number: 364LH. Photodynamic therapy using Lu-Tex induces apoptosis in vitro, and its effect is potentiated by angiostatin in retinal capillary endothelial cells. **Renno R Z; Nelson P S; Holzer R A; Gragoudas E S; Miller J W (Reprint)**. HARVARD UNIV, MASSACHUSETTS EYE & EAR INFIRM, SCH MED, RETINA SERV, LASER LAB, 243 CHARLES ST, BOSTON, MA 02114 (Reprint); HARVARD UNIV, MASSACHUSETTS EYE & EAR INFIRM, SCH MED, RETINA SERV, LASER LAB, BOSTON, MA 02114; HARVARD UNIV, SCHEPENS EYE RES INST, SCH MED, BOSTON, MA 02114. INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE (NOV 2000) Vol. 41, No. 12, pp. 3943-3951. Publisher: ASSOC RESEARCH VISION OPHTHALMOLOGY INC. 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998. ISSN: 0146-0404. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB PURPOSE. To examine the effect of combining angiostatin with photodynamic therapy (PDT) using Lutetium Texaphyrin (Lu-Tex; Alcon, Fort Worth, TX) as a **photosensitizer** in bovine retinal capillary endothelial (BRCE) and retinal pigment epithelial (RPE) cells and to determine the mode of PDT-induced cell death in these cell lines.
METHODS. Cultured BRCE and RPE cells were incubated with angiostatin (500 ng/ml) for 16 hours and subjected to Lu-Tex/PDT, using treatment parameters previously optimized (3 mug/ml Lu-Tex for 30 minutes followed by timed irradiation at 732 nm). Cellular survival was assessed after a

1-week cellular proliferation. Data were analyzed using Student's t-test. Caspase 3 activity was monitored in cells after PDT using a fluorogenic substrate, (Asp-Glu-Val-Asp)-AFC (7-amino-4-trifluoromethyl coumarin) [DEVD-AFC], of caspase 3. After PDT, expression of Bcl-2, Bcl-X-L, Bax, and Bak was also examined in cell lysates by Western blot analysis.

RESULTS. A synergistic cytotoxic effect of angiostatin and Lu-Tex/PDT was observed in BRCE cells at all fluences used (5, 10, and 20 J/cm(2); P less than or equal to 0.05). These findings applied only if angiostatin was delivered before PDT. No such interactive killing effect was observed in RPE cells. Caspase 3 activity was elevated within 10 minutes of PDT in BRCE and RPE cells and was fluence dependent. Differential modulation of Bcl-2 family members was observed after PDT in BRCE and RPE cells.

CONCLUSIONS. The combination of angiostatin and Lu-Tex/PDT potentiates the cytotoxic effect of Lu-Tex/PDT on BRCE but not on RPE cells. This may provide a strategy to increase the selectivity of PDT in damaging capillary endothelial cells with less damage to RPE cells. Lu-Tex PDT induces rapid caspase-dependent apoptosis in BRCE and RPE cells. Furthermore, Lu-Tex PDT induces apoptosis through selective modulation of members of the Bcl-2 family and differs between BRCE and RPE cells.

L24 ANSWER 7 OF 15 CAPLUS COPYRIGHT 2001 ACS
2001:124515 Document No. 134:143907 Photodynamic therapy with verteporfin for **choroidal neovascularization** caused by age-related macular degeneration: results of a single treatment in a phase 1 and 2 study. [Erratum to document cited in CA134:54704]. **Miller, Joan W.**; Schmidt-Erfurth, Ursula; Sickenberg, Michel; Pournaras, Constantin J.; Laqua, Horst; Barbazetto, Irene; Zografos, Leonidas; Piguet, Bertrand; Donati, Guy; Jane, Anne-Marie; Birngruber, Reginald; Van den Berg, Hubert; Strong, H. Andrew; Manjuris, Ulrike; Gray, Todd; Fsadni, Mario; Bressler, Neil M.; Gragoudas, Evangelos S. Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA, USA. Archives of Ophthalmology (Chicago, IL, U. S. , 488 English) 2000. CODEN: AROPAW. ISSN: 0003-9950. Publisher: American Medical Association.

AB Journal omissions of financial disclosure, properly reported at the time of manuscript submission, occurred in the acknowledgment section on page 1171. The following statement should have appeared in the article: "Drs. Sickenberg and Bressler are consultants for CIBA Vision Inc., Duluth, Ga, and QLT Phototherapeutics Inc., Vancouver, British Columbia.".

L24 ANSWER 8 OF 15 CAPLUS COPYRIGHT 2001 ACS
2001:124843 Document No. 134:141913 Photodynamic therapy with verteporfin for **choroidal neovascularization** caused by age-related macular degeneration: Results of retreatments in a phase 1 and 2 study. [Erratum to document cited in CA134:54704]. Schmidt-Erfurth, Ursula; **Miller, Joan W.**; Sickenberg, Michel; Laqua, Horst; Barbazetto, Irene; **Gragoudas, Evangelos, S.**; Zografos, Leonidas; Piguet, Bertrand; Pournaras, Constantin J.; Donati, Guy; Jane, Anne-Marie; Birngruber, Reginald; Van den Berg, Hubert; Strong, H. Andrew; Manjuris, Ulrike; Gray, Todd; Fsadni, Mario; Bressler, Neil M. (Retina Department, University Eye Hospital, Lubeck, Germany . Archives of Ophthalmology (Chicago), 118(4 , 4 (English) 2000. CODEN: AROPAW. ISSN: 0003-9950. Publisher: American Medical Association.

AB Journal omissions of financial disclosure, properly reported at the time of manuscript submission, occurred in the acknowledgment section on page 1167. The following statement should have appeared in the article: "Drs. Sickenberg and Bressler are consultants for CIBA Vision Inc., Duluth, Ga, and QLT Phototherapeutics Inc., Vancouver, British Columbia.".

L24 ANSWER 9 OF 15 CAPLUS COPYRIGHT 2001 ACS
1998:41816 Document No. 128:99370 Angiographic method using green porphyrins in primate eyes. **Miller, Joan W.**; Young, Lucy H. Y.; **Gragoudas, Evangelos S.** (USA . U.S. US 5707986 A 19980113, 9 pp. (English). CODEN: USXXAM. APPLICATION: US 1994-209473 19940314.

AB An angiog. method is disclosed for observation of the condition of blood vessels, including neovasculature in the eyes of living primates, using green porphyrins and light at a wavelength of 600-700 nm to effect fluorescence. Control of exptl. **choroidal neovascularization** using photodynamic therapy with BPD-MA/LDL is described.

L24 ANSWER 10 OF 19 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
1998:241713 Document No.: PREV199800241713. Digital angiography of CNV in the monkey using hematoporphyrin, phthalocyanine and rose bengal. Arbour, J. D.; Connolly, E.; Palmer, T.; **Gragoudas, E. S.; Miller, J. W.**. Mass. Eye Ear Infirmary, Harvard Med. Sch., Boston, MA USA. IOVS, March 15, 1998; Vol. 39, No. 4, pp. S390. Meeting Info.: Annual Meeting of the Association for Research in Vision and Ophthalmology Fort Lauderdale, Florida, USA May 10-15, 1998 Association for Research in Vision and Ophthalmology. Language: English.

L24 ANSWER 11 OF 19 MEDLINE
97221691 Document Number: 97221691. PubMed ID: 9063937. Localization of liposome-delivered benzoporphyrin derivative in the rabbit eye.
Haimovici R; Kramer M; **Miller J W**; Hasan T; Flotte T J; Schomacker K T; **Gragoudas E S**. (Department of Ophthalmology, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston 02114, USA.. CURRENT EYE RESEARCH, 1997 Feb 16 2; 83-90. Journal code: 8104112. ISSN: 0271-3683. Pub. Country: ENGLAND: United Kingdom. Language: English..
AB PURPOSE: Photodynamic therapy (PDT) using the **photosensitizer** Benzoporphyrin derivative monoacid (BPD-MA) or verteporfin is currently under investigation for the treatment of **choroidal neovascularization**. We investigated the localization of the **photosensitizer** using fluorescence microscopy and quantified its presence in ocular tissues after porphyrin extraction using fluorescence spectroscopy. METHODS: Albino rabbits were administered 1mg/kg BPD-MA pre-complexed with low density lipoprotein (LDL) intravenously, or given no treatment. The eyes were enucleated at intervals between 5 minutes and 24 hours after dye injection and were studied with light and fluorescence microscopy, or dissected for porphyrin extraction. RESULTS: At 5 minutes after dye injection, there was bright fluorescence from the choroid and retinal pigment epithelium (RPE) with trace retinal outer segment fluorescence. After 20 minutes, there was increased photoreceptor outer segment and RPE fluorescence but decreased choroidal fluorescence. By 2 hours no fluorescence remained in either the choroid or the photoreceptors and there was diminished fluorescence of the RPE. Trace RPE fluorescence was still visible at 24 hours. Fluorescence localization of liposomal BPD (1mg/kg) at the earliest (5 minutes) time point was indistinguishable from that of the BPD-LDL complex. Using spectrofluorimetry, the highest BPD-MA levels from the eye were measured in the retina-RPE-uvea complex with lower levels measured from the sclera and other tissues. CONCLUSIONS: BPD-MA with LDL rapidly accumulates in the choroid, RPE, and photoreceptors after intravenous injection. Future studies of PDT with BPD-MA for the treatment of fundus disorders may need to address the relationship between dye localization and photodynamically-mediated injury.

L24 ANSWER 12 OF 19 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
97099813 EMBASE Document No.: 1997099565. Photodynamic therapy of exudative age-related macular degeneration. Husain D.; **Miller J.W.**. Dr. J.W. Miller, Retina Service, Massachusetts Eye and Ear Infirmary, 243 Charles St, Boston, MA 02114, United States. Seminars in Ophthalmology 1997 12 (14-5) 1997.
Refs: 57.
ISSN: 0882-0538. CODEN: SEOPE7. Pub. Country: United States. Language: English. Summary Language: English.

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=> s choroidal neovasulature
L31             0 CHOROIDAL NEOVASULATURE

=> s choroidal neovasculture
L32             0 CHOROIDAL NEOVASCULTURE

=> s neovasculature
L33         1026 NEOVASCULATURE

=> s l33 and choroidal
L34           20 L33 AND CHOROIDAL

=> s l34 and age-related macular degeneration
L35            9 L34 AND AGE-RELATED MACULAR DEGENERATION

=> s l35 and treatment
L36            7 L35 AND TREATMENT

=> dup remove l36
PROCESSING COMPLETED FOR L36
L37            5 DUP REMOVE L36 (2 DUPLICATES REMOVED)

=> d l37 1-5 cbib abs

L37 ANSWER 1 OF 5  CAPLUS  COPYRIGHT 2002 ACS
2001:537744  Document No. 135:149263  Methods and compositions for treating
      condition of the eye. Miller, Joan W.; Vrakoudas, Evangelos S.; Renno,
      Reem Z. (Massachusetts Eye and Ear Infirmary, USA). PCT Int. Appl. WO
      2001058241 A2 20010816, 46 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT,
      AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ,
      EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP,
      KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO,
      NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG,
      UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF,
      BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG. (English). CODEN: PIXXD2.
      APPLICATION: WO 2001-US4131 20010209. PRIORITY: US 2000-PV181641
      20000209.
AB    Provided are methods and compns. for the photodynamic therapy (PDT) of
      ocular conditions characterized by the presence of unwanted
      **choroidal neovasculature**, for example, neovascular
      **age-related macular degeneration**.
      The selectivity and sensitivity of the PDT method can be enhanced by
      combining the PDT with an anti-angiogenesis factor, for example,
      angiostatin or endostatin, or with an apoptosis-modulating factor.
      Furthermore, the selectivity and sensitivity of the PDT may be further
      enhanced by coupling a targeting moiety to the photosensitizer so as to
      target the photosensitizer to **choroidal neovasculature**
      .

L37 ANSWER 2 OF 5  BIOSEARCH  COPYRIGHT 2002 ISI (R)
2000:71659  The Genuine Article (R) Number: 317MD. Recent advances in
      photodynamic therapy. Pandey R K  Reprint . NEW YORK STATE DEPT HLTH,
      ROSWELL PK CANC INST, PHOTODYNAM THERAPY CTR, BUFFALO, NY 14263 (Reprint).
      JOURNAL OF PORPHYRINS AND PHTHALOCYANINES (JUN-JUL 2000) Vol. 4, No. 4,
      pp. 368-373. Publisher: JOHN WILEY & SONS LTD. BAFFINS LANE CHICHESTER, W
      SUSSEX PO19 1UD, ENGLAND. ISSN: 1088-4246. Pub. country: USA. Language:
      English.
      *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
AB    Clinical results of photodynamic therapy continue to show promise for
      the **treatment** of various solid malignancies. This paper briefly
      summarizes the advantages/disadvantages of various so-called
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'second-generation' photosensitizers and other medical applications of porphyrin-based analogs. Copyright (C) 2000 John Wiley & Sons, Ltd.

L37 ANSWER 3 OF 5 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
20004[illegible]377 EMBASE Photodynamic therapy with verteporfin for **choroidal** neovascularization. Kaiser P.K., Dr. P.K. Kaiser, Cleveland Clinic Foundation, Desk i3, 9500 Euclid Avenue, Cleveland, OH 44195, United States. kaiserp@ccf.org. Today's Therapeutic Trends 18/4 (313-326) 2000. Refs: 29.
ISSN: 1711-2320. CODEN: TTTRDH. Pub. Country: United States. Language: English. Summary Language: English.
AB Photodynamic therapy (PDT) - administration of a photosensitizing agent which is then activated by the application of a low-intensity light source - is ideally suited for treating **choroidal** neovascularization (CNV), the abnormal development of new blood vessels in the **choroidal** layer of the eye. Verteporfin (Visudyne TM) is the first photosensitizing agent to be approved by the U.S. Food and Drug Administration for use in treating CNV due to **age-related macular degeneration**. Following a 10-minute period of intravenous infusion of verteporfin, its potent photosensitizing effect is efficiently activated by non-thermal light at a longer wavelength than other agents, which allows it to penetrate to a greater depth (5.6 mm). Light activation of verteporfin occurs exclusively within the target **neovasculature**, avoiding any damage to the surrounding delicate ocular structures and the associated risk of vision loss that can occur with thermal laser therapy. Two randomized, placebo-controlled multicenter clinical trials have demonstrated the efficacy and safety of PDT with verteporfin. The first of these, **Treatment** of **Age-Related Macular Degeneration** with Photodynamic Therapy (TAP), confirmed that verteporfin-treated patients had a significantly reduced risk of moderate to severe vision loss after 1 and 2 years of **treatment**. The ongoing Verteporfin in Photodynamic Therapy (VIP) study also showed that patients with subfoveal CNV due to pathologic myopia (a condition for which no previous **treatment** had proven effective) experienced an increased likelihood of vision stabilization after 12 months of verteporfin therapy. This new **treatment** approach represents an important advance in the clinical management of CNV, reducing the growth of **choroidal** neovascular lesions and significantly decreasing the risk of serious vision loss in many affected patients.

L37 ANSWER 4 OF 5 MEDLINE DUPLICATE 1
20011[illegible]2 Document Number: [illegible]797. PubMed ID: 11[illegible]244. Mechanisms of action of photodynamic therapy with verteporfin for the **treatment** of **age-related macular degeneration**. Schmidt-Erfurth U; Hasan T. (University Eye Hospital, Lubeck, Germany.) SURVEY OF OPHTHALMOLOGY, (2000 Nov-Dec) 45 (3) 195-214. Ref: 97. Journal code: [illegible]51. ISSN: 0039-6257. Pub. Country: United States. Language: English.
AB **Age-related macular degeneration**, especially the neovascular form of the disease, is the leading cause of blindness in elderly people in developed countries. Thermal photocoagulation is still the preferred **treatment** for **choroidal** neovascularization that does not involve the fovea, but it is suitable for only a small number of patients and it can lead to immediate loss of visual acuity. Photodynamic therapy with use of photochemical light activation of verteporfin as a photosensitizer (verteporfin therapy) has been shown to be effective in treating vascularized tumors, and its potential to treat other conditions involving neovascularization has also been suggested. Preclinical and clinical studies have indicated that verteporfin therapy can be used to treat **choroidal** neovascularization secondary to **age-related macular degeneration** effectively and

safely. Selective occlusion of **choroidal neovasculature** by this therapy causes minimal damage to the neurosensory retina and, therefore, does not induce l ss of visual acuity. This benefit allows verteporfin therapy to be used in the large proportion of patients who are not eligible for **treatment** by laser photocoagulation. The mechanistic aspects of the mode of action of light-activated verteporfin are described in this review.

L37 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2002 ACS
1999:780978 Document No. 132:332234 Photodynamic immune modulation (PIM). North, John R.; Hunt, David W. C.; Simkin, Guillermo O.; Rakhay, Leslie G.; Chan, Agnes H.; Lui, Harvey M. D.; Levy, Julia G. (QLT PhotoTherapeutics, Inc., Vancouver, BC, Can.). Proceedings of SPIE-The International Society for Optical Engineering, 3863(Biomedical Optics (BMO '99)), 470-474 (English) 1999. CODEN: PSISDG. ISSN: 0277-786X. Publisher: SPIE-The International Society for Optical Engineering.

AB Photodynamic Therapy (PDT) is accepted for **treatment** of superficial and lumen-occluding tumors in regions accessible to activating light and is now known to be effective in closure of **choroidal neovasculature** in **Age Related Macular Degeneration**. PDT utilizes light absorbing drugs (photosensitizers) that generate the localized formation of reactive oxygen species after light exposure. In a no. of systems, PDT has immunomodulatory effects; Photodynamic Immune Modulation (PIM). Using low- intensity photodynamic regimens applied over a large body surface area, progression of mouse autoimmune disease could be inhibited. Further, this **treatment** strongly inhibited the immunol.-mediated contact hypersensitivity response to topically applied chem. haptens. Immune modulation appears to result from selective targeting of activated T lymphocytes and resn. in immunostimulation by antigen presenting cells. Psoriasis, an immune-mediated skin condition, exhibits heightened epidermal cell proliferation, epidermal layer thickening and plaque formation at different body sites. In a recent clin. trial, approx. one-third of patients with psoriasis and arthritis symptoms (psoriatic arthritis) displayed a significant clin. improvement in several psoriasis-related parameters after four weekly whole-body PIM **treatments** with verteporfin. The safety profile was favorable. The capacity of PIM to influence other human immune disorders including rheumatoid arthritis is under extensive evaluation.

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FULL ESTIMATED COST	362.76	362.97

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-25.40	-25.40

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Date: 12/6/2002
Time: 15:21:42

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Inventor Name Search Result

Your Search was:

Last Name = MILLER
First Name = JOAN

Application#	Patent#	Status	Date Filed	Title	Inventor Name
07532859	Not Issued	161	06/04/1990	HAIR CARE SHIELD AND DIVERTER	MILLER , JOAN B.
08942475	Not Issued	168	10/02/1997	USE OF GREEN PORPHYRINS TO TREAT NEOVASCULATURE IN THE EYE	MILLER , JOAN W.
09347382	6225303	150	07/06/1999	USE OF GREEN PORPHYRINS TO TREAT NEOVASCULATURE IN THE EYE	MILLER , JOAN W.
08209473	5707986	150	03/14/1994	AN ANGIOGRAPHIC METHOD USING GREEN PORPHYRINS IN PRIMATE EYES	MILLER , JOAN W.
08390591	5798349	150	02/17/1995	USE OF GREEN PORPHYRINS TO TREAT NEOVASCULATURE IN THE EYE	MILLER , JOAN W.
60114905	Not Issued	159	01/05/1999	TRANS-SCLERAL CONTROLLED-RELEASE DRUG DELIVERY	MILLER , JOAN W.
07950466	5350078	150	09/24/1992	BEVERAGE BOTTLE	MILLER , JOANN H.
07950467	D345506	150	09/24/1992	BEVERAGE BOTTLE	MILLER , JOANN H.
07589384	Not Issued	163	09/27/1990	HUMAN SERUM-BASED CHOLESTEROL CALIBRATORS	MILLER , JOANNE
06459507	Not Issued	161	01/20/1983	COVER UPLIFT	MILLER , JOANNE M.
60044728	Not Issued	159	04/21/1997	CHILD SIZED DOLL	MILLER , JOANNE MARIE
60291340	Not Issued	020	05/16/2001	IMPLANTED MICROMECHANICAL	MILLER, JOAN

				DELIVERY SYSTEM FOR CHRONIC RETINAL DISEASES	
60332200	Not Issued	020	11/21/2001	IMPLANTED MICROMECHANICAL DELIVERY SYSTEM FOR CHRONIC RETINAL DISEASES	MILLER, JOAN
10139656	Not Issued	019	05/02/2002	IMPLANTABLE DRUG DELIVERY DEVICE AND USE THEREOF	MILLER, JOAN W.
09780142	Not Issued	071	02/09/2001	METHODS AND COMPOSITIONS FOR TREATING CONDITIONS OF THE EYE	MILLER, JOAN W.
60349918	Not Issued	020	01/18/2002	METHODS AND COMPOSITIONS FOR PRESERVING PHOTORECEPTOR VIABILITY	MILLER, JOAN W.
09824155	Not Issued	092	04/02/2001	USE OF GREEN PORPHYRINS TO TREAT NEOVASCULATURE IN THE EYE	MILLER, JOAN W.
09478099	Not Issued	041	01/05/2000	TARGETED TRANSSCLERAL CONTROLLED RELEASE DRUG DELIVERY TO THE RETINA AND CHOROID	MILLER, JOAN W.
60181641	Not Issued	159	02/10/2000	METHODS AND COMPOSITIONS FOR TREATING UNWANTED CHOROIDAL NEOVASCULATURE IN THE EYE	MILLER, JOAN WHITTEN

Inventor Search Completed: No Records to Display.

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